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EXPERIMENTS ON THE PERFORMANCE OF PLANKTON SAMPLINGS WITH NET*

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I. Performance Test on Two Types of Plankton Net Flow-Meter Manufactured in Japan

Flow-meters have been devised for use in making plankton net hauls in order to measure the volume of water that is actually filtered by the cloth of the net. In Japan two types of flow-meters have been manufactured and sold (fig. 1). The makers are:

Tsurumi Type: Tsurumi Seiki Kosakusho Co., Ltd.[1)]

Rigosha Type: Rigosha Co., Ltd.[2)]



Fig. 1. Plankton net flow-meters
A: Tsurumi type
B: Rigosha type

Both types were originally designed by Mr. Z. Nakai of the Tokai Regional Fisheries Research Laboratory in Tokyo under a principle similar to that of the Atlas Current Meter,[3)] but smaller in size over-all. They are equipped with a propeller consisting of four blades to convert the force of water flow into revolutions of the shaft which is connected with the dial gauge by a rather complex assemblage of gears. The Atlas Current Meter has been used for oblique haul of a large plankton net, usually one meter in diameter at mouth, and is constructed so as to be very stout, while the instruments in Japan are designed for use in making vertical hauls with a comparatively small net, 45 cm in diameter at mouth. In vertical haul, as compared with oblique haul, the net usually passes through a relatively short distance in the water, hence certain additional revolutions of the propeller of the flow-meter, if they occur as a result of the apparatus being exposed to wind in the air above the sea surface, may result in a higher reading to certain extent on the dial of the flow-meter in relation to the total reading. In addition, in vertical haul a device to stop the counter revolution during descent of the net in the water is necessary. Under such circumstances instruments in Japan are not only small in size in order to correspond with the small size of the net, but also they are equipped with certain devices as stoppers.

The instrument of Tsurumi type is 8.3 cm in diameter and 7.5 cm in height of casing, weighing 0.65 kg in total assembly. It is equipped with two stoppers of very clever device (this part is patented); one stopper posteriorly prevents revolution of the

*水産庁委託対馬暖流調査報告 No. 7

1) 1506, Tsurumi-Cho, Tsurumi-Ku, Yokohama

2) 2, 1-Chome, Kandakaji-Cho, Chiyoda-Ku, Tokyo

3) Atlas Compass and Mfg. Co., Vashon, Washington

propeller during descent in the water by touching with the propeller as it is pressed upwards by water flow from below; the other stopper anteriorly which consists of balance weight and horizontal plate, acts to stop the revolution in the air by means of a stopping pin under the weight. The weight is lifted during ascent in the water because the water flow from above presses the horizontal plate which is set on a bar at the end opposite to the weight.

The instrument of Rigosha type is 6.3 cm in diameter and 5.5 cm in height of casing, weighing 0.4 kg in total assembly. The center hole of the propeller loosely fits to the shaft so as to make it possible for the propeller to slide up and down along the shaft. It has a ratchet on the upper portion of the shaft of the propeller which acts to stop the revolution while the instrument is going down into the depth. There is a weak spring around the shaft just below the propeller. The spring exerts force to lift the propeller up to touch the ratchet, so that the propeller do no longer revolve when the instrument is placed in the air, unless the instrument is faced against the wind of more than about 8 m/sec. But in ascending in the water the propeller is pressed by the water current, unless the hauling speed is less than about 40 cm/sec, and therefore, it is released from the ratchet.

The main purpose of the present experiments is to find out within what range of velocity of water flow these flow-meters show revolutions in constant ratio of the number of revolutions to the distance of tow. Nishizawa & Anraku (1956), when they made a performance test on the flow-meter, Tsurumi type, experienced a certain variation in performance according to individual instruments; some instruments acted very well while others from the same maker did not do so. In the present experiments the best one of four available Tsurumi type instruments was used, but as to the Rigosha type instrument, there was only one available.

Tank Experiments

The experimental tank is 28 cm × 30 cm in section of the water channel, and the water flow driven by the propeller of an electric motor is regulated to keep the same velocity everywhere in the section with the aid of stream-lined plates. The velocity of water flow is recorded by means of a Pitot's tube, but in lower velocity than about 40 cm/sec it was also measured by observing the time required for the drift of a piece of wood for a definite distance. The velocity calculated from Pitot's tube is somewhat less than that calculated from drift of wood when the velocity is lower than about 30 cm/sec, while this is inversely changed at a velocity higher than about 30 cm/sec. It is unclear whether the measurement by Pitot's tube or by drift of a piece of wood shows more accurate value of the velocity, and so in plotting the revolution number of the flow-meters at various velocities of water flow both values of velocities were used. It was impossible to obtain a velocity of water flow higher than about 50 cm/sec.

Both types of instruments, Tsurumi and Rigosha, showed that, when they were placed against the counter water flow in the tank, the revolution of the propeller was immediately stopped by action of the stopper, even at a velocity of water flow as low as 30 cm/sec. This was made by supporting the instruments in horizontal situation; there may be, certainly, some different conditions from case of descending vertically at sea.

Tsurumi type (No. 101) and Rigosha type flow-meter were kept quite horizontal in the experimental tank at a distance just 20 cm below the water surface (from the upper side of casing of the instrument) and to face against the water flow. At various velocities of water flow the time required for 100 revolutions of propeller was recorded (fig. 2). Each experiment at the same velocity of water flow was repeated three or four times, and in most cases the values of revolution showed almost no fluctuation.

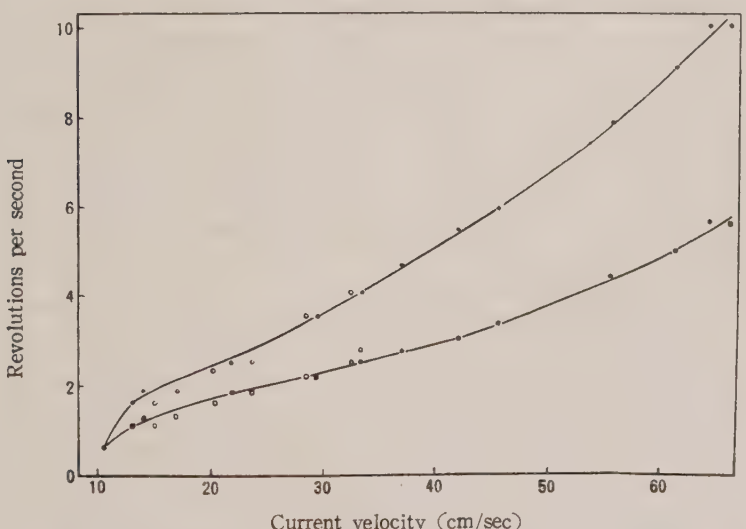


Fig. 2. Performancce of two types of flow-meter in experimental tank
Upper line: Flow-meter, Rigosha Type
Lower line: Flow-meter, Tsurumi Type
• : Current velocity is calculated from Pitot's tube.
○ : Current velocity is calculated from drift of wood piece.

The propeller of neither instrument started to revolve at velocity less than 10 cm/sec, and their performance was very irregular at about that velocity.

Figure 2 shows that the propeller of Rigosha type instrument revolves faster than Tsurumi type, but fast revolution itself does not always mean good performance of the instrument. The two lines in respect of both types drawn for points plotted for the obtained values respectively are both considerably curved (fig. 2). Thus it is indicated that the number of revolutions of two instruments against a definite distance somewhat varies with change of towing velocity, at least in such low velocity as adopted in tank experiments. It must be noticed, however, that the above results might involve the misreading of velocity of water flow in the tank because of inaccurate methods of measurement by Pitot's tube or by drift of wood piece.

Experiments at Sea

Experiments were carried out aboard the "Hokusei Maru" off Hakodate on July 3, 1956, when the sea was rather smooth. The two types of flow-meters were set at the two ends of a plate respectively, which was supported horizontally by an iron bar (fig. 6). This assemblage was lowered into the sea until the wire was run out by 50 meters. Immediately after having been lowered it was hauled up above the sea surface, and the number of revolutions of both flow-meters was recorded. Such experiments were repeated with progressive increase of hauling velocity from 30 cm/sec to 227 cm/sec. The angle of wire suspended to this depth varied from 5 to 15.

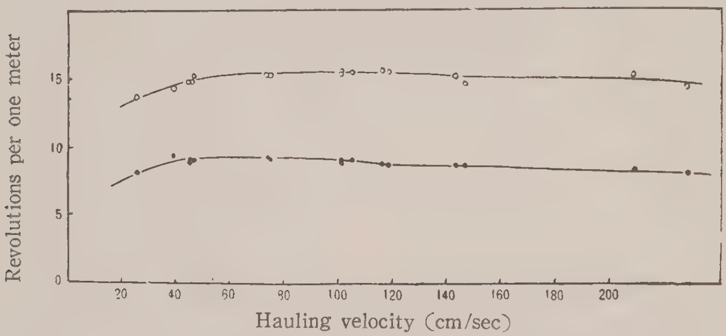


Fig. 3. Performance of two types of flow-meter at sea
• : Tsurumi Type ∘ : Rigosha Type

Figure 3 is a graph of the number of revolutions of flow-meters for each meter at various velocities of haul. It is shown that the revolutions of both types of instrument were kept nearly constant at any velocity except at one less than about 40 cm/sec.

In vertical haul with standard net (45 cm in diameter at mouth) the hauling velocity is usually kept as nearly as possible at one meter per second. In this case the velocity of flow of water that passes through the mouth ring of the net may be less than the above velocity because of resistance offered by the cloth of the net. Although this resistance may be variable with the degree of clogging of the samples on the mesh openings of the cloth, experiments with 45 cm standard net made at sea by vertical haul from 150 meter depth in comparatively clean water showed that revolutions of the flow-meter, Tsurumi type, which was held at the center of the mouth ring of the net, were decreased by about 30-40% as compared with the case of the flow-meter without net (cf. p. 9). It is calculated that the revolution of the flow-meter at the center of the mouth ring of the net occurs as fast as about five times per meter at 120 cm/sec of hauling velocity. These values of revolution correspond to about 60 cm/sec of velocity of water flow; that is, the values are within the limit of performance of the instrument.

II. Estimation of Increase in Hauling Distance in Vertical Haul with Net due to Drift of the Ship

In vertical haul with plankton net, when the ship is not well equipped to adjust her situation against the wind and current, the actual distance of haul would be increased due to the drift of the ship caused by wind (as well as by undersurface current). In such cases the net which is lowered to the desired depth (the length of wire run out is known) would be hauled at some distance from the point at which the net was lowered to the sea surface apart from the original location of the ship, so that the net would pass through a larger distance than the length of wire run out. This increase is included in the reading of flow-meter kept at the center of the mouth ring of the net, and the total amount of water filtered by the net may be simply calculated. However, in the hauls, if flow-meter is not employed, it would yield an error to a certain extent in estimating the volume of plankton in samples. For such reason, usefulness of flow-meter in vertical haul (exactly non-vertical) in bad weather is emphasized (Cushing, 1955).

During the voyage in the Bering Sea in the summer of 1955, a flow-meter Tsurumi type was kept on the special casing on the wire apart from the mouth ring of the net (45 cm × 165cm net) in order to register only the distance of haul irrespective of variation of filtration of the net. Notwithstanding the fact that the length of wire run out was always kept to just 150 meters, the revolutions of the flow-meter held at a such position greatly varied. The variation must have resulted from the changing drifting-rate of the ship, and, therefore, the variation is supposed to have a correlation with the angle of wire when suspended. On more than 70 times of hauls the revolutions of flow-meter are plotted against the angle of wire at the corresponding haul (fig. 4). The curve drawn for distribution of such plottings fits to the formula: $y=0.0275x^2$, where y is increase of hauling distance and x is angle of wire. From this formula the distance of haul is obtained for each round number of degrees of wire (table 1). The present data is based on the hauls of 45 cm × 165 cm net with 10 kg of weight using steel wire of 2.7 mm diameter. The ship "Oshoro Maru" (617 gross ton) is liable to be drifted by wind from the side because of her considerably high free board. The present experiments merely show the necessity of such considerations as increase of hauling distance when the ship is drifted by wind. Investigators should estimate constants of

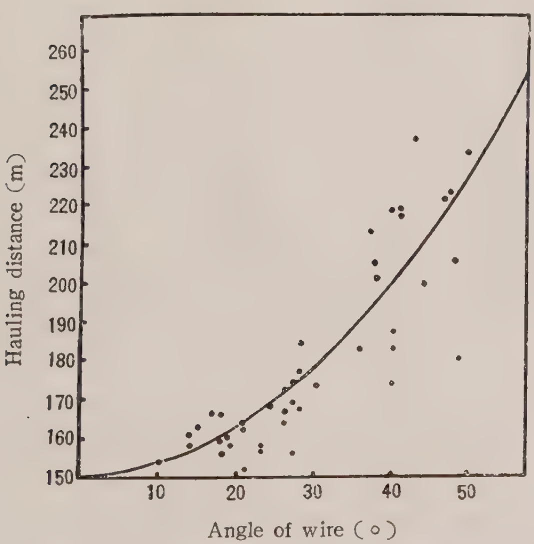


Fig. 4. Relation between hauling distance and wire angle

Table 1. Relationship between wire angle and hauling distance

Length of wire run out (m)	Angle of wire when suspended	Hauling distance calculated (m)
150	0	150
150	5	151
150	10	153
150	15	156
150	20	161
150	25	167
150	30	175
150	35	184
150	40	194
150	45	206
150	50	219
150	55	233

the above formula with their own gears on board their own ships.

On the other hand, if the distance of drift of the ship caused by wind at any velocity can be known by bearing on shore or by direct measurement of current, otherwise empirically, the distance of haul of the net is simply calculated by a formula:

$$x = \sqrt{\alpha^2 + 2dl \sin \alpha + l^2},$$

where x is the distance of haul of the net, α is the angle of wire, d is the distance of drift, and l is the length of wire cast.

III. Estimation of Filtration Coefficient of Various Types of Plankton Net

Most of the works for estimation of filtration coefficient of plankton net have adopted such methods as making comparison of the individual number or volume of plankton organisms between those collected by net haul and those collected quite quantitatively by pumping method or other ways (Kokubo & Tamura, 1931 and many other works). As has been shown, the plankton animals themselves are not always distributed at random even in a limited area at sea (Barnes & Marshall, 1951; Motoda & Anraku, 1955b; Anraku, 1956) and this fact commonly results in a rather wide variation of amount of zooplankton samples in replicate hauls. It seems to be desirable to estimate filtration coefficient of the net more directly, *i. e.*, by physical technique, when zooplankton samples are considered. The present experiments were undertaken for estmation of filtration coefficient of plankton net by reading revolutions of the flow-meter kept on the net.

In vertical haul with net the variation of revolutions of flow-meter which is kept at the center of the mouth ring is supposed to occur because of the varying rate of clogging of the organisms and other suspended materials on the mesh opening of cloth of the net as well as the increasing rate of hauling distance due to the drift of the ship (p. 4). The clogging of the net varies with the abundance and nature of suspended organic and inorganic materials existing at the time of the collection, and the drift of the ship results from the wind pressure against the ship together with the current of water. It is supposed so far as usual ocean collections are concerned, that the variation of

flowmeter readings which is caused by the rate of drift of the ship is exceedingly large compared with that caused by the difference of abundance and nature of materials collected. Clogging of plankton organisms and other suspended materials on the cloth of the net of course may cause a decrease of filtering efficiency of the net, but in the second series of the present experiments successive replicate hauls were made without removing the materials collected in the net. Notwithstanding, the flow-meter readings in replicate hauls with 45 cm net or 20 cm fine mesh net did not yield regular decrease in flow-meter readings. The first haul did not always yield larger number of revolutions of flow-meter than the last hauls (up to ninth). These facts indicate, so far as the case of the second series of the present experiments is concerned, that concentration of materials in the net dces not apparently affect the filtering efficiency of the net. The volume of plankton organisms and other materials collected in this sampling was comparatively small; less than 1 cc for the vertical haul from about 40 meter depth with 45 cm net and also similarly with 20 cm fine mesh net. Accordingly the present data may not be widely adoptod to other cases.

First Series of Experiments

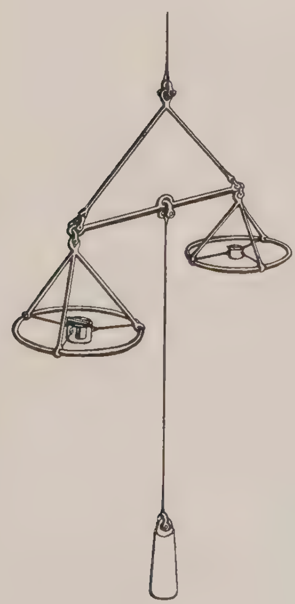


Fig. 5. Assembly of two flow-meters

The first series of experiments was made in the same work as the experiments on the variabilities of catches (p. 15), off Cape Esan, southern Hokkaido, on April 30, 1956. The sea bottom is so deep that the ship was not able to drop her anchor. She was drifted by wind, resulting in a certain extent of inclination of wire when suspended.

Calibration of flow-meter (Exp. 1)

For calibration of flow-meter they were held at the center of a ring of 45 cm diameter, and raised up to sea surface after the wire was paid out by 150 meters. Two flow-meters were hauled simultaneously by assembly in pair (fig. 5, cf. Motoda & Anraku, 1955b). Exact distance of haul was unknown due to the drift of the ship, but supposed increase of hauilng distance was calculated adopting the data on the relationship between the increase of hauling distance and wire angle (p. 5). On this assumption the revolutions of flow-meter for a definite distance of haul at about 1 m/sec of hauling speed were calculated (table 2). The three bridles of the ring as well as horizontal iron rod might have interfered the revolutions of flow-meters to some extent, but the possible error due to this interference

Table 2. Calibration of flow-meter (I)
April 30, 1956, off Cape Esan; "Oshoro Maru", without anchoring

No. of experiments	1	1
Type of flow-meter	Tsurumi 101	Tsurumi 102
Number of hauls repeated	5	6
Mean wire angle (°)	33.5	33.5
Deviation of wire angle (°)	28–38	28–38
Length of wire run out (m)	150	150
Hauling speed (m/sec)	1.1–1.2	1.1–1.2
Hauling distance corrected by increase due to drift of ship (assumed from wire angle) (m)	186	186
Mean revolutions for 186 m	1724	1494
Deviation of revolutions	1425–2086	1268–1865
Revolutions per 100 m	927	803

Table 3. Filtration coefficient of three types of net (I)
April 30, 1956, off Cape Esan; "Oshoro Maru", without anchoring

No. of experiments	2	2,3	3
Type of net	Marutoku Net	North Pacific Standard Silk Net	North Pacific Standard Nylon Net
Flow-meter used	Tsurumi 101	Tsurumi 102	Tsurumi 101
Number of hauls repeated	5	12	5
Mean wire angle (°)	38.4	39.8	39.2
Deviation of wire angle (°)	35–40	30–50	30–50
Length of wire run out (m)	150	150	150
Hauling speed (m/sec)	1.2	1.2	1.2
Hauling distance corrected by increase due to drift of ship (assumed from wire angle) (m)	197	201	199
Mean revolutions of flow-meter	969.8	1056.3	1188.0
Deviation of revolutions	948–1014	982–1145	1125–1240
Revolutions per 100 m	492	525	597
Revolutions in per cent for standard calibration (=filtration coefficient) (%)	53	65	64
Deviation of filtration coefficient (%)	51–55	61–70	61–67

was neglected in consideration. The last line of table 2 gives the number of revolutions of each flow-meter for 100 meter haul. Maximum deviation of revolutions in five or six replicate hauls reached to 21% in Tsurumi 101, and 25% in Tsurumi 102.

Estimation of filtration coefficient of 45 cm net (Exp. 2-3)

Three kinds of nets were used, viz., (1) Marutoku Net, (2) North Pacific Standard Silk Net and (3) North Pacific Standard Nylon Net (p.12). These nets were made of cloth of the same mesh size, approximately 0.33 mm. Assembly of two nets was hauled at the same time. As is shown in table 3, the filtration coefficient of Marutoku Net is 0.53, that of North Pacific Standard Silk Net is 0.65 and that of North Pacific Standard Nylon Net is 0.64.

Second Series of Experiments

The second series of experiments was made in Mutsu Bay from October 29 to November 1, 1956. In most cases the ship dropped anchor, and in the experiments while the ship was drifting the wind was fortunately weak, making it possible to lower the wire almost vertically; thus the increase of hauling distance due to drift of the ship could be neglected.

Calibration of flow-meters (Exp. 4-6)

In this experiment an assembly composed of two flow-meters was hauled without any interference of bridles or wire itself above the meter (fig. 6). Table 4 gives revolutions of four types of flow-meters for 100 meter haul. The largest deviation of revolutions for replicate hauls reached to 3.7% (Rigosha 114), 7.7% (Rigosha, no number), 7.1% (Tsurumi 61), 0.7% (Tsurumi 65), and 9.9% (Tsurumi 102) respectively.

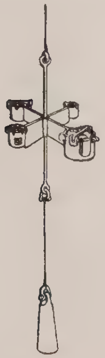


Fig. 6. Assembly of flow-meters for calibration

Estimation of filtration coefficient of 45 cm and 20 cm nets (Exp. 7-10)

The nets were equipped respectively with a flow-meter at the center of mouth ring, and replicate hauls were made separately for each net. Each observed number of revolutions on the net was converted to the number of revolutions for 100 meter haul. From this series of experiments filtration coefficient was estimated to be 0.62 for Marutoku Net and 0.87 for North Pacific Standard both Silk and Nylon Nets (table 5).

Kobayashi and Igarashi (1956) give the following theoretical formula for the filtering-rate of conical plankton net:

$$\sin \varphi_o = \frac{\mu}{\sqrt{1 + \mu^2}}$$

Where $\sin \varphi_o$ is filtering-rate and μ is $\frac{c\alpha}{\sin\theta}$; where c is *Vena Contracta*, α is the ratio of the area of total mesh openings to the total area of net, and 2θ is the angle

Table 4. Calibration of flow-meter (II)
Oct. 30-31, 1956, Mutsu Bay; "Oshoro Maru," anchored

No. of experiments	4	4	5,6	6	5
Type of flow-meter	Rigosha 114	Rigosha no number	Tsurumi 61	Tsurumi 65	Tsurumi 102*
Number of hauls repeated	7	7	8	2	2
Hauling depth (m)	35 or 40	35 or 40	35 or 40	35 or 40	35 or 40
Hauling speed (m/sec)	0.7-1.2	0.7-1.2	0.7-1.2	0.7-1.2	0.7-1.2
Mean revolutions per 100 m	1244	1578	766	690	710
Deviation of revolutions per 100 m	1200-1290	1538-1700	740-820	685-695	640-780

*Instrument, Tsurumi 102, was reconstructed after the experiments in April 1956.

Table 5. Filtration coefficient of four types of net (II)
Oct. 30 - Nov. 1, 1956, Mutsu Bay; "Oshoro Maru," anchored or without anchoring, but wire was suspended nearly in vertical.

No. of experiments	7	8	9	10
Type of net	Marutoku Net	North Pacific Standard Silk Net	North Pacific Standard Nylon Net	20cm × 70cm fine mesh net
Flow-meter used	Tsurumi 61	Tsurumi 61	Tsurumi 61	Tsurumi 61
Number of hauls repeated	9	9	5	5
Hauling depth (m)	28	28 or 40	40	40
Hauling speed (m/sec)	1.1-1.2	1.1-1.2	1.1-1.2	1.5-1.6
Mean revolutions per 100 m	429.7	656.7	670.2	193.5
Deviation of revolutions per 100 m	429-537	550-713	655-690	188-200
Revolutions in per cent for standard calibration (=filtration coefficient) (%)	62	87	87	25
Deviation of filtration coefficient (%)	56-70	72-93	86-88	25-26

of terminal apex of the net. Putting the figures estimated on the materials of the nets used in the present experiments into the above formula the filtering-rate is calculated as 0.8 for Marutoku Net and 0.9 for North Pacific Standard Net. These values are somewhat higher than those obtained in the present experiments to some extent.

Fujita (1956) made a discussion on physical problem of the collection efficiency of a plankton net. Adopting the data, 0.58 and 0.76 of filtration coefficient of Marutoku Net and North Pacific Standard Net obtained by the present experiments, values of constant "α" in his formula (the constant inversely proportional to the viscosity of

water and directly proportional to the magnitude of mesh openings) are calculated with results of 0.86 and 0.94 respectively.

The filtration coefficient of 20 cm × 70 cm fine mesh net (bolting cloth XX 16, 157 meshes per linear inch, approximately 0.086 mm of mesh opening) was 0.25 (table 5).

Nishizawa & Anraku (1956) found that the flow-meter attached at the center of the ring of 20 cm net, and even flow-meter set at the center of 20 cm ring only, revolves faster than the case of flow-meter without ring at the towing speed of 40-80 cm/sec, but the present experiments made at about 1.5 m/sec show only 25% of revolutions of flow-meter on the net for the calibration.

There were found rather high values of filtration coefficients on both Marutoku Net and North Pacific Standard Net in the second series of experiments compared with the values observed in the first series. The difference of values would be caused by non-random samplings whether resulted from technical treatment or from non-random distribution of plankton organisms. At present, the filtration coefficient of Marutoku Net is tentatively supposed to be 0.58 and that of North Pacific Standard Net to be 0.76, taking means of the two series of experiments. This figure is a tentative one, but may be considered as an approximate value in usual case, and may be applied for approximate estimation of zooplankton volumes on the samples collected by non-flowmeter samplings. However, there is a failure in the present experiments, namely, the authors became aware after the experiments that the mesh opening of Marutoku Net used had been decreased from 0.33 mm of original size to approximately 0.21 mm possibly because of clogging during long employment (p.17). The filtration coefficient of new Marutoku Net, therefore, must be larger than the observed data to some extent.

Some of the plankton samplers, *e. g.*, High Speed Plankton Sampler, Gulf I, III (Arnold, 1952; Gehringer, 1952) are equipped with current meter behind the filtering portion; that is, the amount of water filtered by the filtering portion is estimated on the water flow before it runs out from the posterior opening of metal casing of the instrument. It seems more plausible to estimate the water flow at the portion of the flow out because there may be a turbulence of water around the mouth opening to some extent (cf. Currie & Foxton, 1957). How about such estimation on the ordinary conical net in vertical haul ? The following experiments were rather tentative tests on the readings of flow-meters which are attached to the posterior ring of long canvas cylinder containing net.

The revolutions of flow-meters when they are kept at the center of both upper and lower rings of 45 cm × 290 cm canvas cylinder (supposed to be non-filtering) are given in table 6. The lower flow-meter revolves faster than the upper flow-meter by about 1.6. The readings of revolutions (108%) of the lower flow-meter even exceeded the calibrated value. When Marutoku Net is set in this canvas cylinder (fig. 7) the lower flow-meter revolves faster than upper flow-meter by about 2.8 (table 7). In the case of

Table 6. Revolutions of flow-meters kept at the both ends of 45 cm × 290 cm canvas cylinder

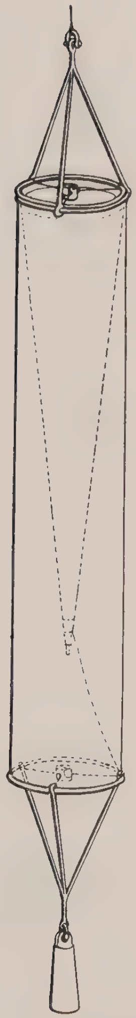
No. of experiments	11	12
Position of flow-meter	Center of upper ring	Center of lower ring
Flow-meter used	Tsurumi 61	Tsurumi 61
Number of hauls repeated	3	3
Hauling depth (m)	40	40
Hauling speed (m/sec)	1.5	1.3-1.4
Mean revolutions in per cent for standard calibration (%)	70	108
Deviation in per cent (%)	69-72	100-109

Table 7. Revolutions of flow-meters kept at the both ends of canvas cylinder installed with Marutoku Net inside

No. of experiments	13	14
Position of flow-meter	Center of upper ring	Center of lower ring
Flow-meter used	Tsurumi 61	Tsurumi 61
Number of hauls repeated	4	3
Hauling depth (m)	40	40
Hauling speed (m/sec)	1.1	1.1
Mean revolutions in per cent for standard calibration (%)	32	90
Deviation in per cent (%)	28-38	88-93

Table 8. Revolutions of flow-meter kept at both ends of canvas cylinder installed with North Pacific Standard Silk Net inside

No. of experiments	15	16	17
Position of flow-meter	Center of upper ring	Center of lower ring	Inner side of lower ring
Flow-meter used	Tsurumi 61	Tsurumi 61	Tsurumi 61
Number of hauls repeated	3	3	3
Hauling depth (m)	40	40	40
Hauling speed (m/sec)	1.1-1.2	1.1-1.2	1.1-1.2
Mean revolutions in per cent for standard calibration (%)	46	88	69
Deviation in per cent (%)	46-46	85-91	64-74


Fig. 7. Canvas cylinder installed with net

North Pacific Standard Net flow-meter set on the lower ring revolves faster than the upper flow-meter by about 1.9 (table 8). The flow-meter set at the center of lower ring of canvas cylinder containing Norpac Net revolves faster than the flow-meter attached on the inside periphery of lower ring by about 1.3, indicating that the water flow in the canvas cylinder is interferred with along the side of cylinder possibly due to the friction of canvas cloth at the side (table 8). When fine mesh net (20 cm × 70 cm) is set inside the 20 cm × 121 cm canvas cylinder, the flow-meter at the center of the lower ring of canvas cylinder revolves faster than the flow-meter at the center of the upper ring by about 1.2 in the case of the smaller Rigosha flow-meter, and by about 2.1 in the case of the larger Tsurumi instrument (table 9).

It is always found that the flow-meter revolves faster at the lower portion than at the upper portion of the canvas cylinder, and, in addition, the flow-meter at the center of the lower ring revolves faster than at the periphery. The revolutions of the flow-meter at the mouth of the net become less when net is covered with canvas cylinder (except the case of 20 cm net) compared with the case of net only. The decrease of water flow due to friction on the inner surface of canvas cylinder might have occurred in this case. The usefulness of canvas cover to obtain more accurate estimation on filtering-rate of the net than usual method cannot be claimed. More detailed experiments and criticism on this problem are expected to be made by physicists.

IV. Specification of "North Pacific Standard Net" and Variabilities of Catches with It

Specification of standard net suggested by Norpac Meeting

Oceanographers and marine biologists from Canada, United States, New Caledonia and Japan had a meeting on North Pacific Oceanography (NORPAC Meeting) in Honolulu in February 1956. The subcommittee on biology of this meeting proposed a recommendation for the standardization of plankton net, depth of collection and quantitative treatment of zooplankton samples in hopes of making truly comparable the data on standing crops of zooplankton from various areas of the Pacific. But it is not suggested that the standard method should necessarily replace the methods now in use which are designed to meet the needs of particular investigations; the standard method may be adopted in addition to those used for special purposes.

Table 9. Revolutions of flow-meter kept at both ends of 20cm × 121cm canvas cylinder installed with 20cm × 70cm fine mesh net

No. of experiments	18	18	19	20
Position of flow-meter	Center of upper ring	Center of lower ring	Center of upper ring	Center of lower ring
Flow-meter used	Rigosha 114	Rigosha no number	Tsurumi 61	Tsurumi 61
Number of hauls repeated	5	5	3	3
Hauling depth (m)	33 or 34	33 or 34	33	40
Hauling speed (m/sec)	0.9-1.1	0.9-1.1	0.7-0.9	1.4-1.5
Mean revolutions in per cent for standard calibration (%)	37	46	24	51
Deviation in per cent (%)	33-42	40-48	23-26	44-55

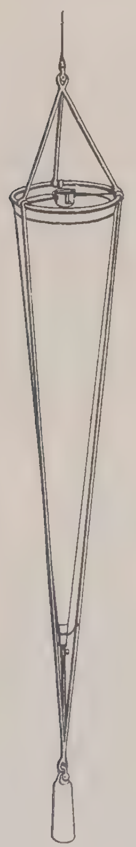


Fig. 8. North Pacific Standard Net rigged for vertical use

Proposed specifications for a standard plankton net (fig. 8) and procedure are as follows:

Net:

Diameter: 45 cm at mouth
Shape: Conical
Length of filtering cloth: 180 cm
Material: 0.33 mm aperture with grit gauze, bolting silk or nylon
Bucket: Not specified

Flow-meter:

A simple, inexpensive meter of small type shall be mounted in the center of the mouth of the net.

Hauling procedure:

The hauls shall be whether vertical or oblique, from an estimated depth of 150 meters. Hauling speed shall be kept 1 m/sec, in maximum 2 m/sec.

Laboratory procedure:

Wet weight determination is suggested. The samples may be weighed after removing water from the organisms by rolling them repeatedly on blotting paper or by any other convenient way. This does not preclude the displacement volume method as well. Exceptionally larger organisms than usual constituents in the samples shall be removed before the measurement.

Reporting of data:

Data on zooplankton concentrations shall be reported as mg or gr wet weight per 1000 cubic meters of water filtered, or as cc in wet displacement volume per 1000 cubic meters.

In the Scripps Institution of Oceanography at La Jolla, California, and the Pacific Oceanic Fishery Investigations in Honolulu customary zooplankton collection have been made by oblique haul with large-sized and coarse-meshed net. They will help to obtain data comparable with those from western Pacific areas mostly operated by Japanese ships by adjusting the sampling in such way that the North Pacific Standard Net is attached on the wire above the customary large net so as to let the standard net sample the plankton through upper 150 meter layer by oblique haul.

In Japan since 1949 most of the plankton investigations supervised by the Fisheries Agency have adopted the method which was standardized by Mr. Z. Nakai for the Cooperative Sardine Research Program. The method is that a conical net, so-called "Marutoku Net", 45 cm in diameter at mouth, 100 cm in length including canvas band, with materials of bolting silk cloth GG 54, 52.5 meshes per linear inch, having approximately 0.33 mm mesh opening, is hauled from the depth of 150 meters as indicated by the length of wire run out, to the sea surface at a speed of 1 m/sec. A small type of flow-meter may be held at the center of the mouth ring of the net to register the amount of water that has been filtered by the net. The modification from this customary net to the "North Pacific Standard Net" is only concerned with the lengthening of the filtering portion.

Japanese ships have usually made collections without concern for the exact depth of haul; the haul has been made for a definite length of wire run out. Although such work is very easy, for comparison of abundance of zooplankton in upper 150 meter layer in various areas the haul should be made from an estimated depth of 150 meters as assumed from the angle of wire. It is desirable, however, when distinct discontinuity of water masses exists within this range of depth that the haul may be made only through that portion of the sea above the depth of such discontinuity, because the principal aim is the comparison of standing crop of zooplankton in the upper water mass.

Diurnal change in zooplankton volume in the upper 150 meters may result in complexity in comparison of abundance of samples over wide area when the samples are collected at different times of the day with each other (cf. King & Hida, 1954; Motoda & Anraku, 1954, 1955a); frequent observations covering day and night, as well as desirably at dawn and twilight, in a comparatively limited area may be able to offer the correction curve.

Analyses of variance of catches by replicate vertical hauls at sea

Because of necessity for comparison of the data obtained hitherto with "Marutoku Net" to those with new standard net in future, the present experiments were undertaken to affirm variabilities of catches with the two types of net and to show the difference between them in amount of total catches. Materials of this experiment are the same collections as used in first series of experiments on filtration coefficient of the nets

(p. 7), made off Cape Esan, Southern Hokkaido, on April 30, 1956. The following three patterns of conical nets were used.

1) 45 cm in diameter at mouth, 100 cm in length, with bolting silk, GG 54 (Marutoku Net)
2) 45 cm in diameter at mouth, 180 cm in length, with bolting silk, GG 54 (North Pacific Standard Net)
3) 45 cm in diameter at mouth, 180 cm in length, with nylon cloth, NGG 54 (North Pacific Standard Net)

Table 10. Replicate hauls with three patterns of net
Ss: Standard Silk Net
Sn: Standard Nylon Net
M: Marutoku Net

No. of haul	Time	Net	Calculated amount of water filtered (m^3)
1	12:46–12:50	Ss M	21.3 17.0 *
2	13:03–13:05	Ss M	21.1 17.6
3	13:16–13:18	Ss M	19.6 16.4
4	13:38–18:40	Ss Sn	21.7 21.5
5	13:48–13:50	Ss Sn	21.3 20.8
6	13:59–14:02	Ss Sn	20.6 21.5 *
7	14:13–14:15	Ss M	20.7 16.6
8	14:30–14:32	Ss M	20.9 16.9
9	14:42–14:44	Ss M	20.3 16.5
10	14:54–14:57	Ss Sn	20.9 19.5
11	15:08–15:11	Ss Sn	22.9 20.3
12	15:18–15:21	Ss Sn	22.3 20.7

*Flow-meter reading failed; assumed from the data of similar wire angle.

The hauls at sea were made according to the procedure shown in table 10; the first three hauls with altogether standard silk net and Marutoku net, the second three hauls with altogether standard silk net and standard nylon net, the third three hauls again with altogether standard silk net and Marutoku net, and the fourth three hauls with altogether standard silk net and standard nylon net.

The amount of water filtered by the nets in each collection is calculated by the revolutions of flow-meters on the net calibrated with non-net flow-meter hauls.

Examining samples in the laboratory the authors became aware of the fact that there happened apparently large number of small-sized copepods and a few other similar sized animals in the samples of Marutoku Net compared with the contents of the samples of North Pacific Standard Net. Such small-sized animals included in the samples of Marutoku Net were 0.8–1.7 gr in a haul, while in the samples of North Pacific Standard Net were only 0.2–0.9 gr. Marutoku Net used in this experiment is that which has long been employed at sea, and later close microscopic examination shows that the mesh openings are clogged with dried adhering materials to some extent, making the mesh opening about 0.21 mm. On the other hand, North Pacific Standard Nets both of silk and nylon used in the experiments were new and the mesh openings of them were about 0.33 mm. These facts might possibly resulted in the finding of the larger number of small-sized animals in Marutoku Net than in North Pacific Standard Net.

Such carelessness in conducting the experiments may much decrease the value of data; in the statistical treatment of the present data small-sized copepods and a few other animals were removed from the analyses. In the five groups of animals individual number and wet weight were estimated separately by groups (tables 11, 12). Analyses of variance were made for the following four series of experiments as to the individual counting only.

Series 1. Three times replicate paired hauls with 45 cm × 100 cm Marutoku Net and 45 cm × 180 cm North Pacific Standard Silk Net

Series 2. Ditto

Series 3. Three times replicate paired hauls with 45 cm × 180 cm North Pacific Standard Silk Net and Nylon Net

Series 4. Ditto

In analyzing the variances, logarithmic values for the number of animals were used instead of the actual number. In the first place, calculation was made as to the logarithms of individual number of main five groups without making correction concerning the filtered amount of water, and eight analyses were made independently. Secondly, the analyses were made regarding the logarithms of individual number after making correction by flow meter readings, *i. e.*, correction for an estimated amount of water filtered, and similarly eight separate analyses were made.

The percentage standard deviation and its 95% fiducial limits calculated from each table of analyses of variances are given in table 13, A and B. Rather wide variation of catches is found in each case. In most cases opposite to expectation North Pacific Standard Net does not yield narrow limits of variation compared with Marutoku Net. The previous report (Motoda & Anraku, 1955b) gives an account of variability of

Table 11. Individual number of five groups of animals in the sample of each haul
(Correction by flow-meter readings is not made)

Series of experiments	1						2						3						4					
Net	45cm × 180cm Silk Net			45cm × 100cm Silk Net			45cm × 180cm Silk Net			45cm × 100cm Silk Net			45cm × 180cm Silk Net			45cm × 180cm Nylon Net			45cm × 180cm Silk Net			45cm × 180cm Nylon Net		
No. of haul	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Calanus plumchrus	1673	1058	723	1409	603	701	739	349	418	544	314	423	408	391	210	546	431	209	222	247	1140	249	244	988
Calanus cristatus	6	4	3	10	4	2	15	1	7	10	5	8	14	6	4	12	1	1	6	7	3	4	10	1
Euphausiids	54	35	20	73	35	15	7	10	34	8	12	45	9	5	3	8	4	2	40	86	75	58	57	81
Themisto	24	11	15	81	54	43	27	18	56	55	30	97	15	66	31	15	24	49	42	61	22	31	16	55
Sagitta	222	99	122	147	71	55	64	72	112	35	84	117	63	94	62	56	92	53	124	117	143	123	106	136
Total	1979	1207	883	1720	767	816	852	450	627	652	445	690	509	562	310	637	552	314	434	518	1383	465	433	1261

Table 12. Wet weight in gr of five groups of animals in the sample of each haul
(Correction by flow-meter readings is not made)

Series of experiments	1						2						3						4					
Net	45cm × 180cm Silk Net			45cm × 100cm Silk Net			45cm × 180cm Silk Net			45cm × 100cm Silk Net			45cm × 180cm Silk Net			45cm × 180cm Nylon Net			45cm × 180cm Silk Net			45cm × 180cm Nylon Net		
No. of haul	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Calanus plumchrus	3.0	2.4	1.8	2.6	2.4	1.7	1.5	0.6	0.7	1.1	0.6	0.7	0.7	0.8	0.3	0.6	0.9	0.3	0.4	0.4	2.7	0.5	0.5	2.4
Calanus cristatus	0.1	0.05	0.05	0.2	0.1	0.05	0.2	0.05	0.1	0.1	0.1	0.1	0.2	0.1	0.05	0.1	0.05	0	0.1	0.1	0.05	0.05	0.1	0.05
Euphausiids	0.1	0.1	0.1	0.1	0.1	0.05	0.05	0.05	0.1	0.05	0.05	0.2	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.1	0.1	0.1	0.1	0.5
Themisto	0.1	0.1	0.1	0.4	0.3	0.5	0.1	0.1	0.3	0.3	0.3	0.5	0.1	0.5	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.3
Sagitta	0.8	0.3	0.6	0.7	0.3	0.3	0.4	0.3	0.5	0.2	0.4	0.4	0.4	0.5	0.3	0.4	0.4	0.4	0.5	0.4	0.7	0.5	0.4	0.6
Total	4.1	2.95	2.65	4.0	3.2	2.6	2.25	1.1	1.7	1.75	1.45	1.9	1.45	1.95	0.9	1.25	1.5	0.95	1.3	1.2	3.65	1.25	1.2	3.85

Table 13. Percentage standard deviations and their 95% fiducial limits (in parenthes) of catches by replicate hauls with 45 cm net

A. Without correction by flow-meter readings

Series of Experiments	45 cm × 100 cm Marutoku Net	45 cm × 180 cm North Pacific Standard Silk Net
1	83 (30-336)	45 (47-212)
2	80 (31-327)	120 (21-487)

Series of Experiments	45 cm × 180 cm North Pacific Standard Nylon Net	45 cm × 180 cm North Pacific Standard Silk Net
3	123 (20-449)	71 (34-294)
4	113 (22-453)	97 (28-362)

B. After correction by flow-meter readings

Series of Experiments	45 cm × 100 cm Marutoku Net	45 cm × 180 cm North Pacific Standard Silk Net
1	81 (31-328)	51 (46-217)
2	82 (30-331)	122 (20-493)

Series of Experiments	45 cm × 180 cm North Pacific Standard Nylon Net	45 cm × 180 cm North Pacific Standard Silk Net
3	120 (21-428)	68 (35-284)
4	98 (25-393)	72 (34-295)

catches by replicate hauls with Marutoku Net to be 47% of percentage standard deviation and 46-217% of 95% fiducial limits, based on the experiments made off Otaru on August 7, 1953. The difference of variation of catches with 45 cm net between the previous and the present experiments is rather large. Difference in population as well as technical inconstancy might have caused this difference of variation of catches. It would be safe to consider that such variation by vertical haul with 45 cm net whether 100 cm long or 180 cm long, has the possibility of reaching the largest values observed, *i. e.*, about 122% of percentage standard deviation ···about 20-493% of 95% fiducial limits. If so, one catch should not be considered as more abundant or less than other catch unless it exceeds the another catch by about five times or reaches one-fifth of other catch in comparison with the mean.

Ratio of catches between 100-cm-long net and 180-cm-long net

For calculating the ratio of catches between both types of the net, the data of the three hauls of the first and second series of the above experiments are grouped together. The calculation gives 1.15 of mean ratio of catches in individual number of 180 cm long North Pacific Standard Net to those of 100 cm long Marutoku Net. Its 95% fiducial limits are 83 and 164%. When the catches are corrected by flow-meter readings (amount of water filtered) these values become 0.92 and 65-152% of 95% fiducial

limits. In wet weight, ratio of catches between the two types of the nets is 0.99 and 71–126% of 95% fiducial limits when the catches are not corrected by flow-meter readings. They become 0.81 and 75–87% of 95% fiducial limits when correction by flow-meter readings is made.

The above results show that the ratio of amount of catches is approximately 1 between 100 cm long Marutoku Net and 180 cm long North Pacific Standard Net, though this ratio varies within considerably wide variation.

It is remembered that the filtration coefficient assumed from flow-meter readings is apparently larger in 180 cm long net than in 100 cm long net (p. 11). Notwithstanding, 180 cm long net does not yield apparently nor statistically larger catch than 100 cm long net. However, such ratio and its variations between two types of the nets are included within the limits of variation of catches in replicate hauls (p. 19).

Duplicate vertical hauls at sea

Our laboratory has used a net of 45 cm × 165 cm, GG 54, 1954. In the Norpac observations to the Bering Sea in the summer of 1955 such net was hauled from approximately 150 meter depth twice at each station (Motoda & Fujii, 1956). The first haul was made before beginning the hydrographic cast and the second haul after finishing that cast. These two samplings for each station very commonly yielded amounts of plankton samples differing from each other to a certain extent.

It is seemingly proven that the frequency of the ratio of half the difference between duplicate samples to the mean of them in displacement volume is distributed normally with the mean value zero (fig. 9), so that the standard deviation of this ratio is calculated with result of 0.250; twice is 0.500, and therefore, 95% fiducial limits are 50% and 150%. In this case volume of one catch should not be considered to differ from that of other catch unless the difference is not larger than the mean of them. The calculation in this case is based on many hauls which were made at various localities covering a wide area, and the materials treated

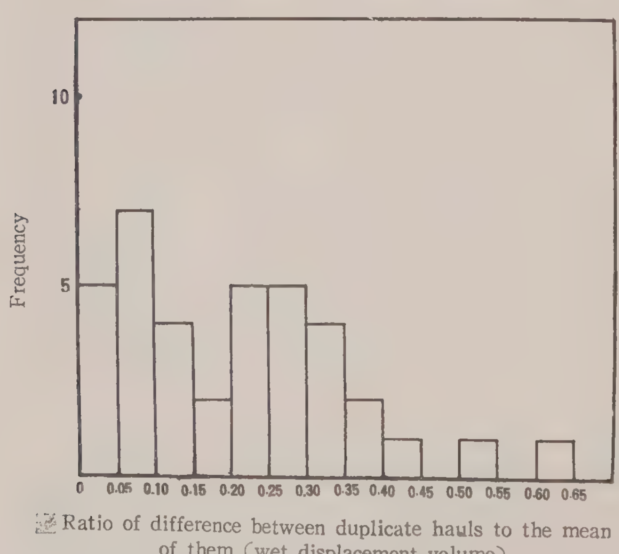


Fig. 9. Histogram showing frequency distribution of ratio of difference to mean of duplicate hauls (half is taken on the abcissa, because a mean is related against lower and upper deviations.)

might have included various different populations.

V. Acknowledgement

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VI. Summary

(1) Two types of flow-meters manufactured in Japan for use of plankton net haul are tested on their performance. In both types of instruments the propeller revolves at constant rate against the unit distance of tow at a speed more than 40 cm/sec. Devices for stopping the revolutions in the air as well as against the reversed flow of water are effectively operated.

(2) Increase of hauling distance in vertical haul of the plankton net occurs when the ship is drifted by the wind. Basing on the experiments at sea for 0–150 meter haul the relation between the angle of suspended wire and distance through which the net has actually passed is given in the formula, $y = 0.0275x^2$, where y is increase of hauling distance and x is wire angle. The constants will vary with the net and weight used.

(3) The filtration coefficient of two types of conical nets, *i. e.*, 45 cm × 100 cm Marutoku Net and 45 cm × 180 cm North Pacific Standard Net, both made of filtering cloth having approximately 0.33 mm of mesh opening, is estimated by reading the revolutions of flow-meter which is attached at the center of the mouth ring, with results of 0.58 in Marutoku Net and 0.76 in North Pacific Standard Net.

(4) Specifications of North Pacific Standard Net recommended at the oceanographic meeting in Honolulu in February 1956 are described.

(5) The variabilities of catches by vertical haul from about 150 meter depth with Marutoku Net and North Pacific Standard Net are calculated on the samples of replicate hauls (table 13). The largest values among the data obtained, 122% of percentage standard deviation with 20–493% of 95% fiducial limits, may be suggested to be adopted on the samples collected with such 45 cm net.

(6) The amount of catches with Marutoku Net and with North Pacific Standard Net is compared statistically; there is no significant difference between them.

(7) Duplicate hauls with 45 cm × 165 cm net made at 37 stations in the Bering Sea show the 0.500 of standard deviation (50 - 150% of 95% fiducial limits) of ratio of half the difference to mean of duplicate catches.

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EFFECTS OF X-IRRADIATION UPON RAINBOW TROUT (*SALMO IRIDEUS*)

I. Influence on the feeding activity in rainbow trout fry*

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As an important symptom of X- and γ-irradiation injury, reports have been published on disturbance of appetite, food intake and water balance in the laboratory mammalian animals such as rat, mouse, rabbit, dog and monkey[1)-15)]. In most of these studies, loss of body weight has been found to be closely associated with the reduction of food intake. Although precise studies in this field have been made using laboratory animals, no information is available on the effects of X-irradiation on fish feeding. In this paper the authors have dealt with changes in the feeding activity of the rainbow trout fry after X-irradiation. Information was obtained by using a revised type of the automatic feeding recorder which has been previously reported by the authors[16)17)]. The present experiment was not only designed for the study of fish feeding, but also for the studies of the effects of X-irradiation on the various organs of the fishes. This paper is the first of series in which it is proposed to present those studies.

The authors wish to express their thanks to the staff members of the Hakodate Kyôkai Hospital, for the use of X-ray facilities. Thanks are also due to Mr. S. Nishizawa of Hokkaido University for his helpful suggestions in regard to expressing the obtained data mathematically.

MATERIALS AND METHODS

A preliminary experiment was performed in October, 1955, though it was not designed for the study of fish feeding. Rainbow trout fry measuring 37–72 mm in total length were used. The radiation factors were as follows: 180 KVP, 3 ma; 0.1 mm Cu filter; target distance 40 cm, including 2.5 cm of the depth of water in a glass dish of 22 cm diameter in which the fishes were contained to be irradiated; diameter of the field size 25 cm; dose rate measured in air 33 r/min. During the periods in which all the irradiation procedure ends, the water temperature was maintained, by addition of ice fragments, at about 12°C which is identical with that of the well-water in the rearing pond. The fishes were divided into five groups, each consisting of 30 – 44 individuals: (1) single exposure, (a) 300 r, (b) 600 r, (c) 1200 r; (2) three times repeated exposures of 200 r with the interval time of a week; and (3) the control non-irradiated. After irradiation these fishes were reared over a period of 115 days in a tank which was equally partitioned into five compartments by fine brass wire screens. The size of each compartment of the tank was 185 cm long, 32 cm broad and 45 cm

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deep. The depth of the water was maintained at about 35 cm, a sufficient volume of well-water flowing into the tank constantly.

As indicated in Table 1, the rainbow trout fry is resistant enough even to the radiation dosage as high as 1200 r. However, even in the 300 r group some distinct effects were found in the reproductive organs, kidney tubules, intestinal epithelium and blood cell constituents[18].

Table 1. Survival of rainbow trout fry after X-irradiation; experimental period: 115 days

Dose r	No. of fish irradiated	Time of death in days (No. of fish died on that day)
300	40	11(1), 14(1), 44(1), 53(2), 59(1), 67(3), 76(1), 101(1)
600	40	11(1), 33(1), 35(1), 59(1), 92(1), 93(1)
1200	44	1(1), 10(2), 17(1), 26(1), 32(1), 35(1), 38(1), 39(1)
200 × 3	30	6(2), 12(1), 28(1), 54(1), 62(1)
Control	40	11(1), 14(1), 15(1), 45(1), 72(1)

In view of the results of the preliminary experiment, the exposure of rainbow trout fry to low dosage of X-ray was tried again on August 10, 1956, to ascertain its effects on the organs above noted, together with changes of the feeding activity. The fishes used were 2550 in number, which had been artificially fertilized and reared for about three months in a small tank of well-water set in a greenhouse of the Faculty of Fisheries. The average values of the body length and body weight of 12 fish sampled at random at the time of irradiation were 42–57 mm and 1.3–3.5 g. All of the fish were divided into three groups, two for exposure to 100 r and 500 r, and the third the non-irradiated control. The radiation factors were almost the same as those of the preliminary experiment, except that throughout the periods of X-irradiation procedure the water containing the fish was gently bubbled by supplying oxygen. After irradiation the three groups of fish were transferred for rearing into an out-door concrete pond which was partitioned into three compartments by fine brass wire screens. Out of the three groups of the irradiated and control fishes, 30 each individuals of uniform body weight were carefully selected and transferred into the equally partitioned respective compartments of a tank set in a greenhouse which was designed for the feeding experiment. The size of each compartment was 185 cm long, 53 cm broad and 45 cm deep. As in the preliminary experiment well-water was flowed into the tank constantly, to maintain the depth at about 35 cm. Feeding records were taken continuously more than 100 days after irradiation, though all the data are not presented in this paper. To maintain the water quality in good conditions throughout the periods of X-irradiation procedure, the fishes were fasted for about 24 hours before irradiation. After irradiated, the fishes to be used in the feeding experiment were transferred into their respective

compartments of the tank and further fasted for about 20 hours there. So, these fishes were first supplied bait after about 44 hours, and then, the feeding records were taken.

The principle of construction of the feeding recorder is identical with that of the type previously reported[16,17]. That is, the bait is suspended in a tank of water to make the fish peck it. By the pecking of the fish the bait box vibrates, and so the slender aluminum bar which is set vertically to the box swings like a pendulum and touches the horizontally placed aluminum bar; then the electric current is closed, and thus, a feeding mark is recorded on the kymograph by a magnetized lever. The parts of contact in both slender aluminum bars are plated especially with silver. The bait was prepared mincing the following materials: boiled intestine of domestic animals, boiled potato, fresh beef liver, dried crustacean larvae and rice-bran. The bait was changed twice a day for fresh material at about 9 a. m. and 7 p. m. which correspond to the times when the active feeding of the fishes terminates temporarily considering from their diurnal rhythm. Further, it was previously ascertained that disturbance of the feeding by the exchange of the bait is slight as reported in the experiment with the goldfish[17].

RESULTS

General observations

Besides the experiments for the sake of the feeding record, some observations were made on the behaviour of both irradiated and control fishes reared in the out-door pond. The most noticeable behaviour of both groups 100 r and 500 r treated, was that they appeared to be in an inactive state during a period of more than one week following irradiation. The pond is partitioned longitudinally into the three compartments, each size being of 445 cm long, 110 cm broad and 100 cm deep. The depth of the water was maintained at about 75 cm, well water flowing into the respective compartments separately. Near the outflow side of the pond, a transverse partition of fine brass wire screen is set to be common to the three longitudinal compartments. All the irradiated fishes of each group were found to be crowded in a mass so as to cause their bodies to adhere to the corners of the transverse partition, while the control fishes were observed to swim about vigorously except the first day on which they were transferred into this pond. It was often observed that some irradiated fishes swam out towards the center of the compartment against the current, but they returned immediately to the original situations as if frightened at some phenomena occurring in the upper stream of the pond. Possibly this unusual behaviour might be in part due to depression of their swimming activity following irradiation. If this is true, and further, if any materials contributing to the buoyancy of their bodies were absent, the irradiated fishes might be drifted away by current.

Depression and recovery of the feeding activity

To learn the daily standard deviation of the feeding frequency within the groups, a test was made on the three groups of control fishes for ten consecutive days in the feeding experiment tank and also in the other small tank. From these experiments, it was found that coefficient of variance in the daily feeding frequency is of the order of 12 – 26 % if the individual fishes are selected to be of uniform body weight.

The feeding frequencies of the irradiated and control groups are given in Table 2. To determine the approximate day number following irradiation on which its influence is statistically significant, comparison of the daily difference in the feeding frequency was made between the control and each irradiated group for each three adjacent days in

Table 2. Daily feeding frequency post-irradiation

Days Post-irradiation	Feeding frequency: Control	100 r		500 r	
1	113+	74+		233+	
2	87	69		73	
3	332	173		39	
4	211	74	*	41	*
5	299+	72+	*	37+	*
6	369	101	**	33	*
7	233	34		16	*
8	212	168		14	**
9	217+	133+		27+	*
10	190	57	*	93	*
11	131	48	*	20	
12	232+	117+		34+	*
13	193	175		29	
14	166	110		93	*
15	214	43		64	*
16	298+	145+		177+	**
17	326	278		210	
18	381	441		120	
19	182+	276+		40+	*
20	317	236		132	*
21	420	248		146	
22	369	284		329	
23	264	243		431	
24	311	236		200	
25	359+	422+		187+	

The mark + means that the recording was interrupted in part in the course of the experiment on that day.

*, ** see the explanation in text.

a series, i. e., *days 1–2–3*, *2–3–4*, *3–4–5*, etc. In this table, an asterisk indicating statistical significance was put on each middle day of the three adjacent ores tested; for instance, if the test on the feeding frequency of *days 3–4–5* showed to be significant more than 95% of probability, an asterisk was put on *day 4*. As the test was made by such a method, for instance, the feeding frequency on *day 1* of the 500 r group is not shown to be significant though it might be highly significant actually. The percent changes of the feeding frequencies in the irradiated groups from the control are illustrated in Fig. 1. The general pattern of the irradiation effects on the feeding activity will be easily seen from this figure.

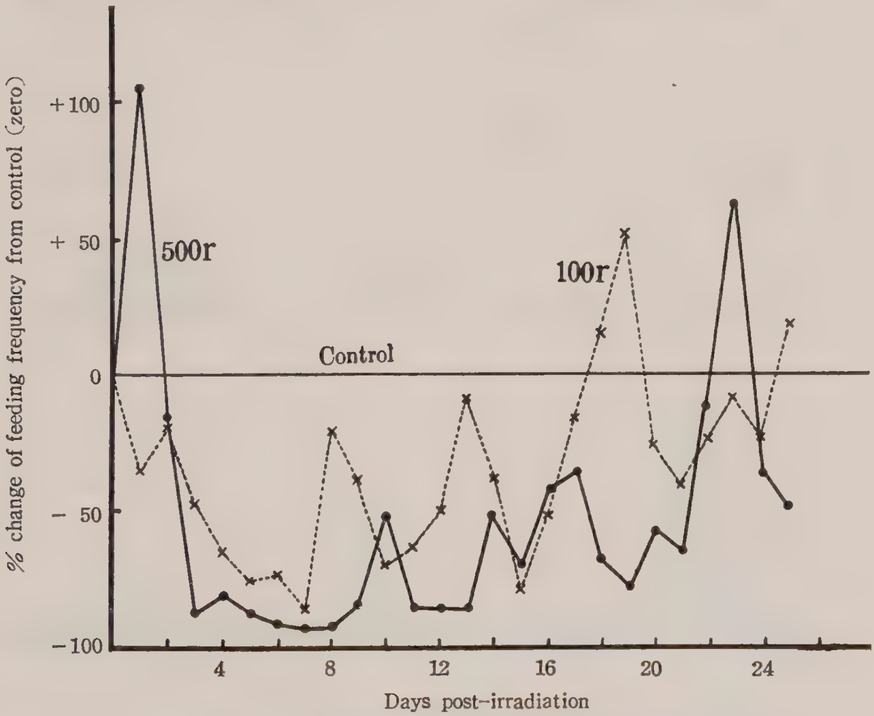


Fig. 1. Percent change of the feeding frequency in the irradiated fishes from the control (zero); 500 r •——• and 100 r ×······×

The feeding records of the 500 r group were begun 18 hours after irradiation. Consequently, although the changes in the period immediately after irradiation are not available here, it is presumable from the records of Fig. 2 that the relatively marked increase of the feeding in this group began to occur in the beginning of the afternoon of *day 1*, namely within 24 hours after irradiation. It is noteworthy that such a supracontrol feeding was recorded on *day 1* in the 500 r group. A severe depression of feeding was found first to occur on *day 3*, showing −88% from the control. Thereafter, the depression continued until *day 9*, with the range of −81∼−93% from the control.

First recovery occurred on *day 10* with -51% from the control. The second depression continued during the period of *days 11-13* with a magnitude of about -85%

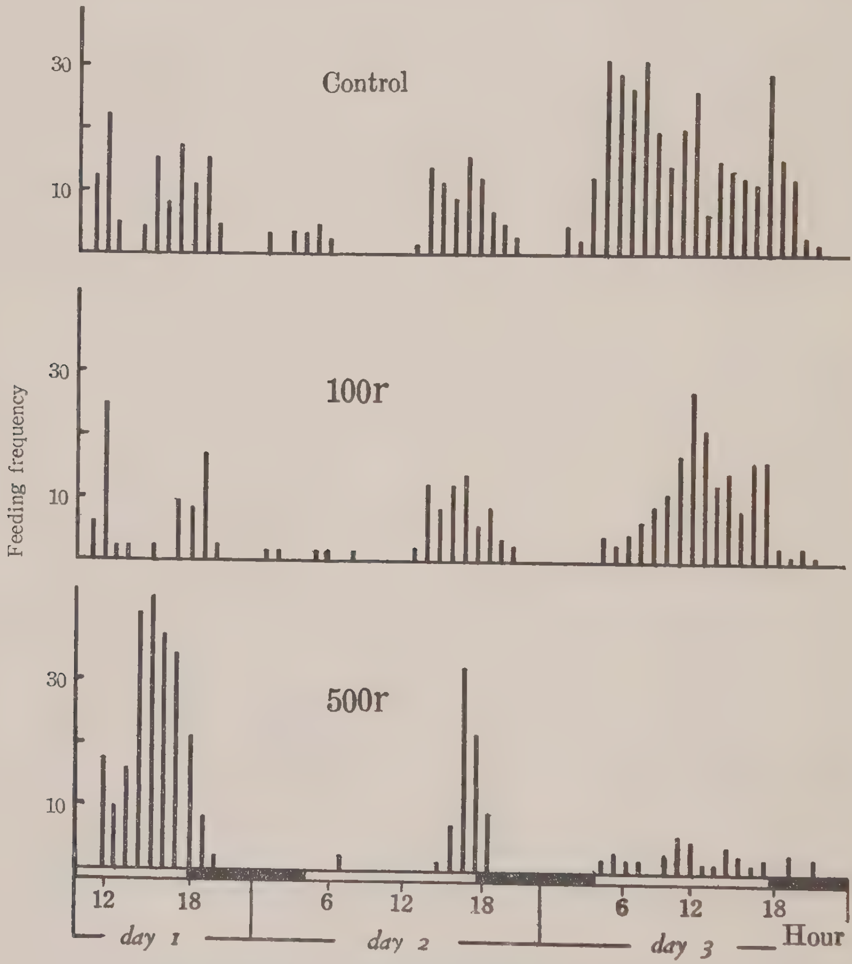


Fig. 2. Diurnal rhythms of the feeding activity in the three groups: control, 100 r and 500 r, in the period of *days 1 - 3*

from the control. The second recovery was found to occur on *days 14-17*. This recovery may be said to be relatively marked as is also seen in Table 2. After the slight depressions following this second recovery, a very distinct recovery was recorded on *days 22* and *23*. The feeding frequency of the latter day was far beyond the control level. On the basis of these observation it can be said that the feeding activity of the 500 r group almost returns to the normal level by about 3 weeks after irradiation, though the recovery is not yet complete.

In the fishes of the 100 r group, the inhibition of the feeding activity was far

slighter in comparison with that of the 500 r group. However, the irradiation effects were clearly found as is shown in Fig. 1 and Table 2. That is, the feeding frequency declined with a slow declivity until *day 7*. The first but very distinct recovery occurred on *day 8* with the feeding 79% of the control. Although the second depression continued during the period of about *days 10* and *11*, the feeding activity almost returned to the control level on *day 13*.

Diurnal rhythm of the feeding activity

Correlation between the diurnal rhythm of the feeding activity in the rainbow trout fry and the environmental factors will be studied in future. Here, only the effect of X-irradiation on the rhythm of the said activity is analyzed. To show the general appearance of the effect, the results of *days 3–22* were illustrated in Fig. 3, A – D, dividing these days into the four periods, *days 3–7*, *8–12*, *13–17* and *18–22*. In these figures, the diurnal rhythm of the feeding activity in each group is expressed as the hourly average in each period of the days above cited. From these figures, the recovery process in the diurnal rhythm of the said activity will be clearly seen, together with the fact that following irradiation the rhythm is not so severely distorted as the activity itself is highly affected. Such a relationship will be judged also from the rhythms illustrated in Fig. 2. It is clearly seen from Fig. 3 that the feeding activity in the control group shows a distinct diurnal rhythm. Especially, the rhythms shown in Fig. 3, B and C may be said to be typical. Namely, the two peaks of the feeding activity are found daily, one at daylight and the other before sunset. Of these two peaks, the latter one occurs invariably, usually with a higher activity than the former. Generally saying, the feeding begins actively towards daylight, then decreases gradually until about 9–11 a. m., thereafter increases at the beginning of the afternoon, and reaches the peak in the time before sunset. The said activity in the night-time is usually very feeble, at least, during the present experimental period. As is clear in Fig. 3, the diurnal rhythms of the irradiated groups are found in general to be closely parallel to that of the control. The rhythm of the 500 r is shown to be indistinct in the first period of *days 3–7*. However, it will be seen from Fig. 2 that the relatively active feedings occur in the peak time of evening although the feeding activity itself is severely depressed. In the second porid of *days 8-12*, the peak in the time of evening becomes distinct even in the 500 r group. The diurnal rhythm in the 100 r group appears to be almost identical to that of the control, though the feeding frequency is not yet so. No difference of the diurnal rhythm of the feeding activity was found between the two groups of 100 r and 500 r in the third period of *days 13–17*. Consequently, so far as only the diurnal rhythm is concerned, it may be said that 500 r group almost returned to normal in this third period. In the last period of *days 19–22*, the rhythms of all the three groups are

almost the same, though the feeding frequency in the 500 r group is apparently still depressed slightly.

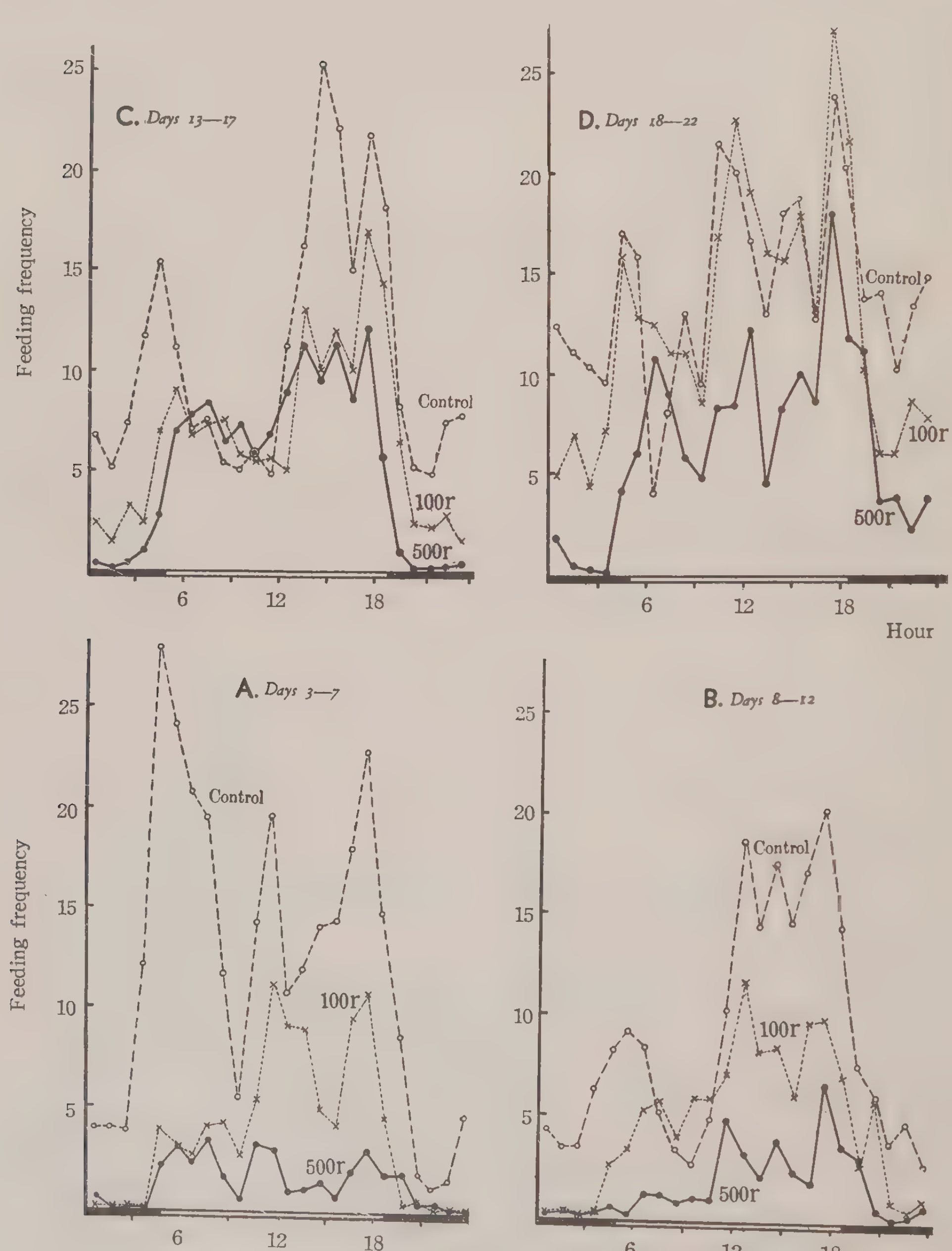


Fig. 3. Comparison of the diurnal rhythms of the feeding activity in the three groups: control, 100 r and 500 r, illustrated as the hourly average of each period of *days 3–7* (A), *8–12* (B), *13–17* (C) and *18–22* (D)

DISCUSSION

Susceptibility of the gastrointestinal tract to X- and γ-irradiation has been well established on the laboratory mammalian animals[1)15)]. Although no literatures concerning the effect of X-irradiation on fish feeding were available here, it is noteworthy that the present findings on the rainbow trout fry were in general agreement with those which have been reported by the previous workers on the mammalian animals.

Supracontrol feeding was found to occur in the 500 r group of the fishes on *day 1* after irradiation. It seems that such a relatively marked increase of food intake following irradiation has not been reported in the other animals. It has been generally recognized that following irradiation a marked increase of water consumption occurs in rats on *day 1* and often does again after several days, for instance, when food intake returned to normal[4)-6)]. Although further experiments are necessary before usual occurrence of supracontrol feeding in the irradiated fishes can be definitely asserted, it is biologically of much interest when considered from the facts that the experimental animal used is an aquatic one, fish, and also that such an increase was found only in the fishes irradiated with a relatively higher dosage, similar to the case of the water consumption in rats[7)].

It has been reported that the threshold dosage of X-ray for eliciting anorexia and loss of body weight is as low as 25 r or 50 r in rats[6)]. So, it may not be a surprising fact that in the rainbow trout fry a slight depression of the feeding activity was found following the exposure of 100 r. The magnitude and duration of the inhibition in the feeding activity after irradiation is also in agreement with the findings in the mammals, being dependent upon the radiation dosage to which the fishes were exposed. In most of the mammalian animals, the initial change of the gastrointestinal tract such as decrease of food intake, anorexia, increase of intestinal motility and delayed gastric emptying activity has been found to occur immediately after or as early as 24 hours or so after irradiation. Being different from the terrestrial animals, in the case of the aquatic fishes the effects of handlings such as netting, transfer and transport of them for irradiation must also be taken into consideration, in addition to the effect of irradiation itself. So, it seems that in the present experiment various kinds of stresses resultant from the handlings might affect the fishes in many ways[19)20)]. Swift *et al.*[10)] have emphasized that gastric emptying in rats is found also to be delayed significantly by some stress-producing treatments other than radiation. In fact, the feeding activity of the control group in the present experiment was found to be distinctly depressed by the handlings until *day 2*. Analysis of the initial change of the feeding activity in these fishes is difficult as stated above. However, considering from the records of the said activity taken 18 – 22 hours after irradiation, it can be said that marked changes relative to the control group occurred first on *day 1* and *day 3* in the 500 r group and on *day 4*

in the 100 r group.

Similarly as in the case of the mammalian animals, the recovery of the feeding activity in the irradiated fishes was found to occur exponentially, accompanying on the graph with a wavy process of repeated decreases and increases. If the wavy fluctuation on the way to recovery is neglected, it seems that the recovery of the feeding activity occurs approximately corresponding to the exponential curves calculated from the following equation:

$$R=-ae^{-kt} \quad \cdots\cdots\cdots\cdots\cdots\cdots \quad (1)$$

where R means magnitude of change of feeding frequency from the control (zero) or magnitude of recovery, a magnitude of the greatest inhibition, t day number in the recovery process or t_0 the day showing the greatest inhibition, and k means recovery coefficient.

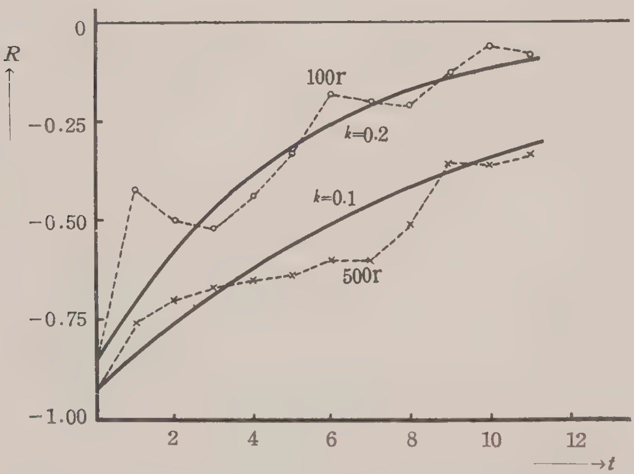


Fig. 4. Recovery curves of the feeding activity experimental and calculated; t_0 corresponds to *day 7* in the 100 r group and to *day 8* in the 500 r group

In the present experiment, the greatest inhibition of the feeding activity occurred on August 17, *day 7* in the 100 r group, and on August 18, *day 8* in the 500 r group, the depression from the control showing −0.86 and −0.93 (−86 % and −93 %) respectively. The curves of the solid lines in Fig. 4 were obtained introducing these values into a, and 0.2 in the 100 r group and 0.1 in the 500 r group into k, of equation (1). Those of dotted lines show the moving 7-day average obtained from the differences of the feeding frequency from the control (cf. Fig. 1). It can be said that in both groups of 100 r and 500 r the two curves experimental and calculated are in agreement with each other, respectively. That is, the approximate process of recovery of the feeding activity in the two groups of rainbow trout fry after irradiation may be inferred respectively from the following equations:

$$R=-0.86e^{-0.2t} \qquad (100\ r)$$

$$R=-0.93e^{-0.1t} \qquad (500\ r)$$

If the recovery coefficient, k, is widely utilizable as a characteristic in the study of X-irradiation injury, approximate recovery process of some function or response in a

given animal irradiated may be expressed succinctly by it.

It seems to be important from the fisheries point of view that inactive state of the fishes continued for at least several days following irradiation. This is true because, if similar phenomenon occurs widely in fishes, those which have suffered some radiation injury might be drifted far away by current during the period of their inactive state.

The fact that no difference in the number of dead individuals was found beween the control and 1200 r groups seems to show that rainbow trout fry have a greater resistance to X-irradiation than goldfishes have[21)-24)]. This greater resistance may be associated with the low water temperature in which these cold water fishes were reared, similar to the findings in infant mice that the lowerd environmental temperature decreases the mortality following irradiation[25)]. However, the problem of survival rate in relation to low temperature appears to be somewhat complicated in the study of X-irradiation[25)26)].

SUMMARY

1. Using an automatic recording apparatus, the effect of X-irradiation on the feeding activity was studied in the rainbow trout fry.

2. In the 500 r group, a supracontrol feeding was recorded on the first day following irradiation. The marked depression of feeding occurred first on the third day and continued until the 9th day, the change in this period showing a range of -81～-63% from the control. On the 22nd day, the feeding activity of this group almost returned to the control level, followed by a wavy process of repeated decreases and increases.

3. A slight depression of the feeding was found in the 100 r group. A relatively marked depression occurred first on the 4th day after irradiation, showing -65% from the control. The depression continued until the 7th day, on which the greatest inhibition of -86% from the control was recorded. Feeding returned to the control level on the 13th day, accompanying also with a wavy process on the way of recovery course.

4. Diurnal rhythm of the feeding activity in the irradiated fishes was not so severely distorted as the said activity itself was highly affected. Consequently, the recovery of the former occurred a little earlier than that of the latter.

5. Exponential recovery process of some function or response in a given animal irradiated will be given by the following equation: $R=-ae^{-kt}$; where R means magnitude of change of feeding frequency from the control (zero) or magnitude of recovery, a magnitude of the greatest inhibition, t day number in the recovery process or t_0 the day showing the greatest inhibition, and k means recovery coefficient. On the basis of this idea, approximate process of recovery of the feeding activity in the irradiated rainbow trout fry will be inferred from the following equations: $R=-0.86e^{-0.2t}$ in the 100 r group and $R=-0.93e^{-0.1t}$ in the 500 r group.

6. Following irradiation the fishes of both 100 r and 500 r groups showed an inactive state for at least several days. This fact seems to be important from the

fisheries point of view, because fishes which have received some radiation injury might be drifted away by current during the period of their inactive state.

7. It was found that the rainbow trout fry are resistant even to the radiation dosage as high as 1200 r. It can be said that this fish has a greater resistance to X-irradiation than goldfish has.

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BLOOD CELL CONSTITUENTS IN FISH

I. Peroxidase Staining of the Leucocytes in Rainbow Trout (*Salmo irideus*)

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It is well known that cellular changes in the constitution of the human blood occur in delicate response to any failure of the state of health, and that fluctuation of such values has been utilized universally for the diagnosis of diseases. Dawson has reported that a similar relationship is found also in lower vertebrate animals. According to his studies on lead-poisoning in mud-puppy[1] and catfish[2], much more satisfactory evidence relative to health has been given by its injurious effects on the circulating erythrocytes than by the chemical detection of this metal in both the tissues and body fluids of these animals. Further, he has found in a study on 20 species of marine fishes that a significant correlation exists between the ecological condition of each species and the value of relative proportion of both immature and mature erythrocytes[3].

Thus, it seems that fluctuations of the blood cell constituents are utilizable as a biological indicator in the study of fish physiology. However, that the studies based on this idea have never advanced in this field may be attributed to the fact that the identification of blood cells in this animal is not a little difficult. The difficulty is considered to be partly due to the fact that undifferentiated blood cells are usually found to flow in the blood stream even in the normal condition of fish. It has been reported that such a state resembles that of hemopoiesis at the begining of the third stage of the fetus in the higher vertebrates[4].

In comparison with the cells of the erythrocytic series, the identification for those of the leucocytic series in fish is difficult. Especially, among the cells of the leucocytic series in fish blood, discrimination between the granulocyte and monocyte is considerably difficult, and further, it is much more difficult to distinguish the young cells of both monocyte and granulocyte from the cells of lymphocytic group. Further, as Werzberg[5] and Chûin[4] have observed, the morphological features of the granulocytes in fish are highly multifarious but almost specific according to the species or family. The descriptions of the monocytes in fish which have been reported by previous workers are in disagreement. It seems possible that such confusion is caused by difficulty in the classification of young and transitional cells in the monocytic group. For instance, a large discrepancy has been found among the ratios of the granulocyte, monocyte and lymphocyte in the blood stream of the crucian carp examined in winter[6,7]. Such a discrepancy may have been brought about mainly by the following two causes. Namely, one is the personal difference raised by taking a subjective view in the classification of undifferentiated blood cells which are difficult of identification; the other is the ecological

difference such as habitat of the individual fishes to be examined, which brings about variations of the blood cells as reflected in their physiological states.

It is the author's opinion that it is very difficult to settle the cause of such a discrepancy only by application of the Romanowsky staining methods. It is essentially desirable that some staining method by which the blood cells are clearly and easily discernible from one another is employed, if one wishes to utilize changes of the ratio in the leucocytic constituents as a biological indicator in fish physiology. Therefore, on the basis of this idea the present author examined the various tests of both peroxidase and oxidase reactions which have been tried in human blood to distinguish myeloic leucocytes from lymphatic leucocytes. From these tests it was found that a modification of Sato & Sekiya's copper method[8] shows a good result for the purpose stated in the above. By the application of this method to 18 species of fishes, it was clarified that it is not only available on the blood of the rainbow trout, but also on that of these other fishes. Using mainly the blood of the rainbow trout, an establishment of the standardization was made in the method for measuring the constituent ratio of the groups among the leucocytic series.

Before proceeding further, it should be stated that much of this work was suggested by Prof. Shinjiro Kobayashi, of Hokkaido University, to whom the author owes thanks for his constant guidance and help rendered during the course of this work, and for his kindness in the revision of the manuscript. The author is also indebted to Assist. Prof. T. Kubo of Hokkaido University, who aided in supplying the materials used. Thanks are likewise offered to Prof. S. I. Sato and Mr. K. Kobayashi of Hokkaido University for kindly identifying the species of the fishes used. The author wishes further to acknowledge his indebtedness to Mr. M. Mogami for kind assistance.

MATERIALS AND METHODS

Most of the rainbow trout used in the present experiment were five or six years old, which were reared in a pond of the Hatchery of the Faculty of Fisheries, at Nanaye, near Hakodate.

A fresh, dry blood smear was prepared by a routine method, using blood taken from the caudal vessels of the fish. For preparing the smear, the two methods of Wright-Giemsa's stain[9] and Sato & Sekiya's peroxidase reaction were used. Although several methods were tested to obtain the positive reactions for both peroxidase and oxidase of the blood cells, no satisfactory result was shown by the following methods[9]: Schultze, Pappenheim, Graham, Bloch & Peck, Goodpasture, and Osgood. However, the positive reaction could be obtained clearly by a slight modification of Sato & Sekiya's method described below. Besides this, the Nickel method devised by Mitsui & Ikeda[10] also gave a good result for the reaction concerned.

Sato & Sekiya's method modified in the present investigation is as follows.

Immediately after the fresh, dry blood smear was applied to 1 % solution of copper sulphate, a small quantity of 0.1 % benzidin solution was poured on it. In every use of the last reagent, a few drops of 3 % hydrogen peroxide were added to it to be about 0.04 %, and then, the mixture was warmed to 30° – 40°C. After the smear was applied for 1 minute to the warmed solution, it was washed with water to remove the blue particles precipitated. Next, it was counterstained for 2 minutes with 1 % solution of safranin.

OBSERVATIONS

Peroxidase reaction in the leucocytes

In the peroxidase-stained smear of human blood prepared by Sato & Sekiya's method, cytoplasm of the myeloic leucocyte is stained blue or green producing "positive reaction", while the remaining cells of the blood other than these myeloic leucocytes show "negative reaction", only being coloured red with the safranin of the counterstain. Hollande[11] has stated on the granules stained green or blue by this method that they are to be called "oxybenzidinophilic granules", because peroxidase, an enzyme, which is included within the blood cell, by catalysis oxidizes the reagent used, and then, the so-called peroxidase-granules are stained secondarily by the benzidin blue which is produced by the oxidation.

Similarly as in the case of human blood the peroxidase-positive leucocytes were observed also in the blood of the rainbow trout (Pl. I, Fig. V). As is clearly seen in these figures, there are found the green or blue granules such as above noted within the cytoplasms of these cells. The nuclei are stained by the safranin of the counterstain. In most cases, however, the shape of the nucleus is not clearly observable owing to the presence of the stained granules which are densely distributed within the cytoplasm.

In the peroxidase-negative leucocyte, neither blue nor green granules were found, but the cell was stained red as a whole by safranin and its nucleus was coloured more deeply than the cytoplasm was (Figs. VI–VIII). Although not illustrated in this paper, the cells of the erythrocytic series showed also the negative reaction in the peroxidase-stain; nevertheless, fine granules were often recognized in these cells. However, as the general appearance of the granules in the cells of the erythrocytic series is clearly different from that of the positive leucocyte, it was easy to distinguish them from each other. Further, the granules of the erythrocytic series appear to be quite different in size from those which have been observed in the frog blood.[12]

Peroxidase staining and Wright-Giemsa staining

As stated in the former section, by means of the peroxidase-stained smear the leucocytes of the rainbow trout are classified into the two categories with an exceedingly high contrast, one group showing a positive reaction and the other group a negative

reaction. However, it is a fault in this method that the morphological features of the positive leucocytes are not clear being covered almost wholly by the dense distribution of the stained granules though those features of the negative ones are easily observable. Fortunately, this fault could be remedied by a comparative observation of the leucocytes stained by the peroxidase reaction with those stained by Wright-Giemsa's method. The series of cells in Figs. I–IV show the general features of the leucocytes in the rainbow trout which were stained by the Wright-Giemsa staining. Judging from their morphological characteristics, the cells shown in Figs. I and VI appear to belong to the same group corresponding morphologically to each other. This group is considered to consist of the cells which belong to the lymphocytic group. The groups of cells shown in Figs. II and VII are regarded as spindle cells, both of them corresponding to each other. The morphological appearance of both lymphocytes and spindle cells described above are in general agreement with the descriptions of the previous workers.[2,4,5] Thus, if the two methods, the peroxidase stained smear and the Wright-Giemsa stained smear, are employed in parallel for staining the leucocytes, a morphological identity will be found between the two groups of the figures obtained. For instance, it becomes clear that the series of cells in Figs. III and IV are identical with those in Fig. V which showed the positive reaction in the peroxidase stained smear. In the series of cells shown in Figs. III and IV, neither granules as stained by the acidic dyes nor by alkaline dyes are found in the cytoplasm. However, the cytoplasm of the cells shown in Fig. III appears blue being relatively deeply stained by alkaline dye, while that of the cells in Fig. IV is stained light blue. In the cells of the former group, the shape of the nucleus is either somewhat rounded or slightly invaginated in part or bilobed though incompletely. The nuclei in the cells of the latter groups are either rounded or rod-like in shape in most of the smaller cells, but those of the larger ones are usually either rod-like or lobated into more than two parts. From these characteristics, it is considered that the two series of cells shown in Figs. III and IV belong respectively to separate groups. Judging from the observations of the previous workers,[4-7] the cells shown in Fig. III possibly belong to the monocyte of the leucocytic series. As stated above, there are not found any granules in the cells shown in Fig. IV. However, the nuclei of these cells resemble those of staff cells and segmentocyte which have been found in human blood.[13,14] In addition to the Wright-Giemsa staining, a few methods belonging to Romanowsky's staining system were tried on such cells as shown in Fig. IV to ascertain whether granules are to be found within them or not. Although all these attempts failed to reveal any granules, the author has assumed those cells to belong to certain group of the granulocyte, considering from their specific morphological features.

Basophilic and eosinophilic granulocytes have been observed in the blood of many fishes similar to those in human blood.[4-7] However, Werzberg[5] has reported the

occurrence of a group of the leucocyte without granules in some fishes, besides the lymphocyte and monocyte which similarly do not possess the granules. The cells shown in Fig. IV are considered to be identical with the type which has been observed by Werzberg. Owing to the further description involved, the author herein has called these cells "specific granulocyte" temporarily.

Fading of the stained granules in peroxidase positive reaction

It seems that the specific granulocytes and monocytes shown in Figs. III and IV are identical with the cells which reacted positively to the peroxidase-stain. Such an assumption will be supported by the fact described below. When the peroxidase-stained smear is left, for instance, for a few weeks as it was mounted with Canada balsam, the blue or green granules lose their characteristic colours and become brownish materials of diphenoquinonediimine. In such a faded smear, the nucleus becomes clearly visible within the cell. Thus, it is clarified that the general appearance of the cells is in agreement with that of cells stained by Wright-Giemsa's method.

Peroxidase-reaction in young leucocytes

The cells with lobated nuclei such as shown in Fig. III, 5,6 and Fig. IV, 6-12 are considered to be mature forms of the monocytic and granulocytic groups. All these cells were figured after it had been made certain that both groups of these cells are identical with each other through a comparative observation of the smear preparations faded from the peroxidase-positive stain to the diphenoquinonediimine as described in the former section. Besides these cells, immature ones belonging to the leucocytes are found in the blood stream of the fish. However, it seems to be very difficult to distinguish these immature cells from those of the other series of the blood elements. For instance, the series of the cells, especially, the apparently immature ones as shown in Fig. VIII, 1 - 3, resemble morphologically the monocytic ones, while those showed the negative reaction to the peroxidase staining. However, although as in the case described above it is very difficult to classify the cells of the leucocytic series, it is very easy to discriminate them from each other according to the peroxidase-positive and -negative reactions. With regard to the questionable cells, however, it seems that further experiments are necessary to establish the author's idea presented in this paper.

Application of the peroxidase-stain to the other fishes

Using the modification of Sato & Sekiya's method, the peroxidase-positive reaction was clearly recognizd in the circulating blood of the following teleost fishes similarly as in the rainbow trout: marine fishes — atka mackerel, *Pleurogrammus azonus*; starry flounder, *Platichthys stellatus*; 2 species of rockfish, *Sebastes taczanowskii*, *S. thompsoni*; 2 species of puffer, *Fugu vermicularis porphyreus*, *F. pardalis*; wingfish,

Lepidotrigla microptera; dace, *Tribolodon hakonensis hakonensis*; masu, *Oncorhynchus masou*; freshwater fishes —— carp, *Cyprinus carpio*; crucian carp, goldfish, *Carassius auratus*; medaka, *Oryzias latipes*; anadromous form and fry of chum salmon, *Oncorhynchus keta*; dark parr and smolt forms of masu, *O. masou*; smolt form of malma, *Salvelinus leucomaenis*; eel, *Anguilla japonica*.

Kawamoto[15] has reported that the positive leucocytes were not found in eel blood though he tried using the peroxidase reaction of Freisch's method. However, the author could clearly observe them in the eel blood using a modification of Sato & Sekiya's peroxidase staining. In eel blood, there was found a different type of leucocyte containing a few granules stained blue. The occurrence of these two types of leucocytes in the eel appears to be a characteristic for the blood of this fish which is different from that of the other fishes examined.

DISCUSSION

The fact that the morphological features of the granulocytes in fish blood are multifarious but almost specific according to the species or family, has been reported in detail by many workers.[4)-7)] In the rainbow trout, there was not found any leucocyte which contains granules stained with either alkaline or acidic dye, though "specific granulocyte" which has none of the granules was observed. A similar observation has been reported in fish by Werzberg.[5)] That is, out of 14 species of the marine and freshwater fishes studied, in 5 species he has found the leucocytes without granules which belong to neither monocyte nor lymphocyte. While Chûin[4)] has reported the occurrence of the granulocytes in all of the 24 species of marine fishes which he studied; he has stated that as the granules of granulocyte in fish are very unstable the fixation of them is difficult, and so, much care in their staining is necessary. The author examined the presence or absence of the granulocyte containing the granules in the blood of 18 species of fishes by Wright-Giemsa's staining, which is the same method as used in the case of the rainbow trout. In consequence of this investigation, it was ascertained that the granulocyte without granules was to be found only in the salmonid fishes studied, viz., in anadromous form and fry of chum salmon, both marine and freshwater forms of masu, malma, and rainbow trout; also it was ascertained that in the blood of all the other fishes studied the granulocyte occurs with granules, which are stained with the dyes acidic and alkaline or either of them. Accordingly, it may be said that the author's observation that the granules were not found in the granulocyte of the rainbow trout is due to neither the staining reagent nor the method used.

Even in the granulocyte in which the granules are easily recognized, colouration of them is not clear in the young cells of this group. Cytoplasm of the young cell has such a common characteristic as to be stained by methylene blue, an alkaline dye.[9 13 14)]

So, the discrimination is difficult between the young granulocyte and young monocyte stained by such an alkaline dye, and it is much more difficult to distinguish the two sorts of young cells from those of the lymphocytic series. A similar relationship occurs also in the case of the specific granulocyte (granulocyte without granules). If the purpose of observation is restricted only to the hemocytological study, perhaps discrimination between them may be possible by means of, for instance, some excellent methods other than Romanowsky's such as vital staining or supravital staining. However, if one would study the fish blood purposing to make its constituents a biological indicator, it is essential that the staining method be simple but sure. As to this point, it was found that the modification of Sato & Sekiya's method used in the present observation is available for use on the 19 species of both marine and freshwater fishes. As the blood smear prepared by this method shows highly contrasted stainings of both the peroxidase-positive and -negative reactions, the relative proportion of the cells reacted to them is easily measurable. In this case, the positive leucocytes are made up of both the monocytic and granulocytic groups which are indistinguishable from each other by this peroxidase-staining. However, so far as the mature cells of these groups are concerned, they can be discriminated from each other by a comparative application of Romanowsky's method and the peroxidase staining.

It may be said that one weak point of the peroxidase-staining is that the classification of young cells is impossible owing to the occurrence of their multifarious types in the blood stream of fish. However, even if it is not possible to discriminate the monocytic group from the granulocytic one by this method, both of them showing the positive reaction are easily distinguishable from the other groups of the leucocytes which show the negative reaction. Consequently, the peroxidase reaction is useful for analysis of the leucocytic constituents as a biological indicator. That is, if the leucocytes which have peroxidase, an enzyme, are considered to be a unit of the cells, fluctuation of their occurrence in fish blood may be utilized to judge any change of physiological states in fish if it has happened.

SUMMARY

1. The peroxidase reaction by which discrimination has been made between myeloic and lymphatic leucocytes in human blood, was applied to the circulating blood of the rainbow trout.

2. To obtain the positive reaction in both the peroxidase and oxidase stainings, the following methods were tried, but none of these gave any satisfactory results in reaction: Schultze, Pappenheim, Graham, Bloch & Peck, Goodpasture, and Osgood.

3. Sato & Sekiya's peroxidase reaction showed a good result in its slight modification as follows. Although the reagent used was the same as that of the original method, the benzidin solution was warmed at about 35°C and the staining was made at the time

when the benzidin had dissolved completely. So, green or blue granules were clearly seen within the cytoplasm of the peroxidase-positive cells.

4. Upon comparison of the result of the positive reaction in this peroxidase staining with results obtained by Romanowsky's staining, it was found that the peroxidase-positive cells included both monocytic and granulocytic groups.

5. So far as the mature cells are concerned, monocyte is distinguishable from granulocyte by Romanowsky's staining. However, identification of the young ones of these cells is difficult, and further, it is much more difficult to discriminate between these young cells and lymphocytic cells. In the peroxidase staining, as both the monocyte and granulocyte show the positive reaction, those groups are easily discernible from the lymphocytic cells and from the other blood elements all of which show the negative reaction.

6. Although by the present peroxidase staining, it is impossible to make distinction between monocytic and granulocytic cells, if both of them are considered to be a unit of the cells showing the positive reaction or containing the same enzyme, peroxidase, fluctuation of the ratio measured between the positive and negative cells seems to be utilizable in fish physiology as a biological indicator.

7. The peroxidase staining was applied to 18 species of fish; pertinent results were obtained similarly to the case of the rainbow trout.

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PLATE I

Explanation of Figures

The figures show the general feature of the leucocytes in the circulating blood of the rainbow trout.

Figs. I, II, III, and IV show the leucocytes stained by Wright-Giemsa's method.

Figs. V, VI, VII and VIII show the leucocytes stained by the modification of Sato & Sekiya's method, V showing the positive reaction and VI – VIII the negative reaction.

The cells shown in Figs. I and VI belong to lymphocytic group, and those of Figs. II and VII to the group of spindle cells.

The cells in Fig. V which showed the positive reaction contain the green or blue granules and correspond to those of Figs. III and IV which were staind by Wright-Giemsa's method. The cells in Fig. III belong to the monocytic group, and those of Fig. IV to the granulocytic group.

Classification of the cells shown in Fig. VIII is not clear at present.

All of these figures were drawn with the aid of an Abbe's transcriber.

還 流 水 槽 の 試 作

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Trial production of a circulating-water tank

Tetsuo ÔKAWA

Abstract

In order to make it possible to conduct model experiments on fishing implements or ships the writer prepared a small circulating-water tank. Its form is an open channel having rectangular section as shown in Fig. 1 and Fig. 5 (Right). One half horse power electric motor, stepless torque converter and four blade screw are equipped to circulate the water in the tank.

In order to get the stream-line flow as uniformly as possible, guide vanes shown in Fig. 3 are set at three corners (C, E, G in Fig. 1) and five aerofoil blades shown in Fig. 4 are equipped in front of the second corner (D in Fig. 1).

For the purpose of making sure that the current speed is uniform at the measuring section of this tank, the writer adjusted the stream line with the guide vanes observing by means of twenty-five pitot tubes as shown in Fig. 5 and then got the range of uniform current speed from 0 cm/sec to 50 cm/sec as shown in Figs. 6-9.

ディプレッサー・プランクトンネットなどの漁具や，船舶等の各種模型の流水試験を便ならしめるために，小型の還流水槽を試作したのでその構造の大略を発表する。

I　全 体 構 造

全体の構造は第1図の平面図に示してある。Mは誘導電動電導機（3相交流）で $\frac{1}{2}$ HP，プーリーは3吋φ，廻転数は毎分1420である。Rは無段変速機で，これはモーターから平ベルトで駆動される。プーリーは10吋φ従つて426R. P. M.。内部構造はVベルト式であるため無段変速し，その変速比は4：1である。Tは水槽本体で図の如く各隅にトタン板で $\frac{1}{4}$ 円周の円味をつけ，更にタンクの内部にトタン板で塀をくみ水路を形成する。水路は矩形開溝でその断面は285mm×300mmで，これに260mmまで水を入れる（第5図参照）。従つて水路は略楕円形となり，外側にて長軸1m655mm短軸1m145mmである。各隅の曲率半経は内側で100mm，外側で385mmである。

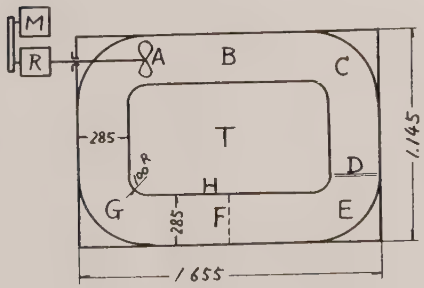


Fig. 1 Plane figure of the circulating-water tank

外側壁及び底部は板囲して之にトタン板を張つたゝめに充分な強度があるが，内側壁は水圧のために倒れるおそれがあるのでそれを防止するため内部にも水を入れておく。

II　各　　部

1. スクリウー：第1図にてAがスクリウであるが，無段変速機（R）に直結して廻転し流速を起す。真鍮板（厚さ1.2mm）製で直径225mmφ の4枚羽根からなり，タンクの外壁に設けたグランドを通る軸に取付けてある。変速機変速比によりスクリウーの廻転数が変り，それによつて流

速が変ずる。

2. 格子：水はスクリウーによつて押し出されるために，その流線は捩れている。それを直線流にするためにこの格子をおく。この寸法は第2図に示すが，格子目の大きさは50mm角（トタン板28＃製）である。

この格子を配置することによつて，流速の捩れは防止され，更に測定個所（第1図F）の流速はこれを置かない場合より早くなる。配置個所はスクリウーから押し出される流速の早さにより違えなければならないが，開溝の上面の流速を見ながら流速の捩れが略その中央に来ると思われる個所（第1図B附近）にその前端をおくとよい。この配置個所を変えることによつて測定個所の流速は変り，又水路内の各部の流速にも変化がある。この格子は水流におされて流されぬようにセットする。

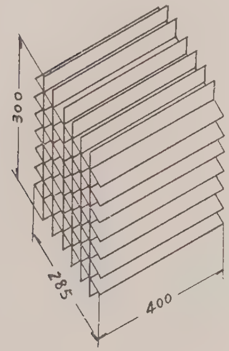


Fig. 2 Latticework

3. 整流板：第3図の如き整流板を第1図のC・E・Gの3隅におく。格子を通つて来た流水はその慣性力のために曲線路に来ると外側が早くなり，且つ外周に水が盛り上つてくるのでこれを防止し，更に曲線路の内側にも適当な流速を与えるためにこの整流板をおくのである。但し低速流の場合にはGの位置を除いた2隅だけにしても大して差障りはない。図の如く5枚（最外側のは外壁に代用）のトタン板（28＃）を間隔50mmに底飯び及び側板にハンダ付して支える。

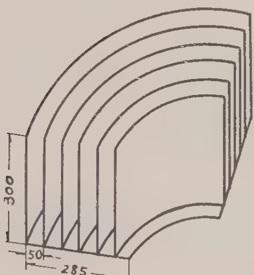


Fig. 3 Guide vanes

4. 流速調整装置（翼）：前記整流板をおくことによつて溝の内側に適当な流速のあるように努めたが，これだけでは内側と外側の流速を同一にすることは仲々困難である。従つてこの内外の流速を調整するために第4図の装置をおいた。

この流速調整装置5枚を第2隅（第1図E）の整流板の前方（同D）に之と直接に接触しておき，蝶番で之に取付け手可動式に調節操作の出来るようにする。これらのアタックアングルをいろいろ変化させることによつて，第2の整流板の各溝内に入り込む水流を調節して測定個所における流速を一様ならしめようとするものである。

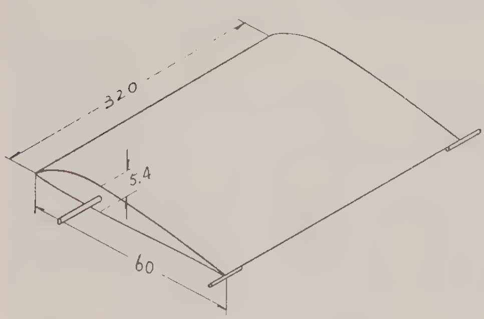


Fig. 4 Aerofoil blade

流速調整装置の断面は翼型N. A. C. A. 4309で，翼幅60mm，翼長320mm（水中260mm），翼厚5.4mm，従つて水中のアスペクト比は48である。翼型にした理由は，水の渦流を出来るだけ少なくするためであるが，翼型にするとその厚みだけ水路の有効断面積を絞ることになる。翼型形式をN.A.C.A.4309にしたのは他の形式のものに比して肉の厚さが最薄で流水の絞りが最小（水流有効断面積最大）のためである。

5. ピトー管群：模型試験を行うべき測定個所の流速を一様ならしめるために翼型の流速調整装置を操作して調節を行うのであるが，一枚の翼を動かすことによつてそれに直接関係がないと思われるような離れている溝の内部の流速にも変化が起きてくる。このために1本のピトー管で測定個所断面の流速を測定し乍ら断面が一様な流速になるように調整することは非常に煩瑣であり且つ時間もかゝる。

これを簡単に且つ早く調整を完了するために25本のガラス製動圧測定ピトー管群を流速測定流域内に縦横に等間隔に配列した。この25点の流速をゴム管で導いて直管内の水頭で現わし一度に比較観測をしつゝ翼型

のアタックアングルを加減しながら流速を調整するのである。尙，この動圧を導いた直管内の水位は低くて読みにくいので25本を1本のやゝ太めの管に導いてこの中の空気を或程度抜いて水位を高くし（目測の高さ）読み易くした。

Ⅲ 測　　定

以上のように，25点に於ける動圧を同一水頭になるように調節し終つた後でこれを取去り，然る後キャリブレーションの出来ているピトー管でその25点の流速を測定したものが第6図〜第9図である。平均流速としてあらわした数値は25点のうち外壁及び底に近いものは壁面の影響が考えられるので中央部の9点をとり，この数値の代数和平均をとつたものである。

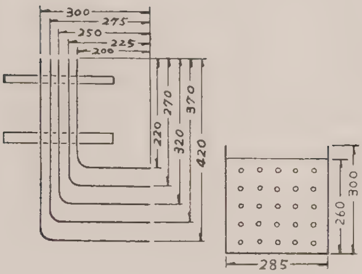


Fig. 5 Twenty-five pitot tubes

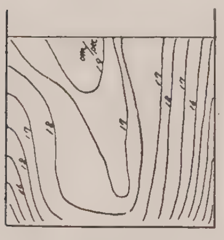


Fig. 6 Speed distribution chart (1) 17.3 cm/sec

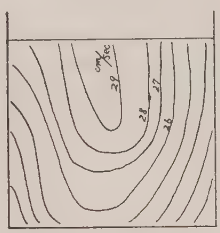


Fig. 7 Speed distribution chart (2) 27.8 cm/sec

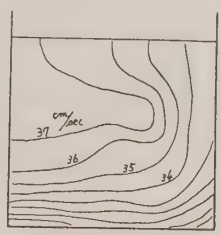


Fig. 8 Speed distribution chart (3) 36.3 cm/sec

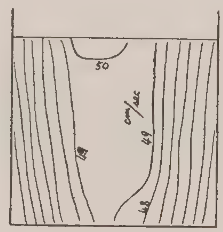


Fig. 9 Speed distribution chart (4) 49.1 cm/sec

Ⅳ 検　　討

1. データーにみられる如く，速度分布は略良好で，使用断面範囲は壁面の摩擦等の影響のないと思われる水路の中央部約 180mm × 160mm を適当とする。そして水面からの深浅による流速の変化はないようである。

2. この寸法の装置では試験を行い得る範囲は約80cm（第1図Eの後端よりGの前端まで）である。

3. 流速が大になつてくると，壁面の微少の凹凸が流線に影響を与え，水面に波が立つてくる。それまでの速度流域は0 cm/sec 〜 50cm/secである。勿論開溝の内壁の凹凸を少なくすれば流速は更に大きく出来る。

4. 速度はピトー管で測定する時には静動水頭差の2乗根に比例して効いてくるが，流速が非常に小さい時はガラス管群の互の水頭差が小さくて読みにくく従つて調節しにくい。

5. 翼型の幅は水路の内側に於けるものの方を長くした方が内外同一の長さにしたものより流線調節に楽である。

6. 水泡はスクリウー附近では生ずるが観測点附近では殆どなく，又渦流も殆ど生じない。

7. 第1図のHはこの部分だけを板ガラスとしてある。その内部に必要に応じて角形のガラス函を沈めその中に鏡を45°に設置して上部より観測し，又はカメラをおいて水中の模型を撮影しようとするものである。

尙，本試作に終始御指導をいたゞいた小林喜一郎教授並に五十嵐脩蔵助手に厚く感謝する次第である。

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プランクトンネットの濾水率について（Ⅱ報）

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On the filtering-rate of plankton net

Shûzô IGARASHI

Abstract

The author, in a previous report, got the next formula $v/V=\mu/\sqrt{1+\mu^2}$ concerning the filtering-rate of plankton net. Upon the towing of the plankton net in the sea, plankton will fill the mesh of the net so that the filtering-rate will be decreased. In this report, the author studied the decrement of the filtering-rate of plankton net on a few assumptions and got formula (5) and its numerical graph Fig. (2). Fig. (2) shows the relation between v, α and t, and means that the current speed inside the net is decreased with the lapse of towing time, so the filtering-rate is also decreased and also that the higher the towing speed is, the greater is the rate of decrement of the filtering-rate.

ま え が き

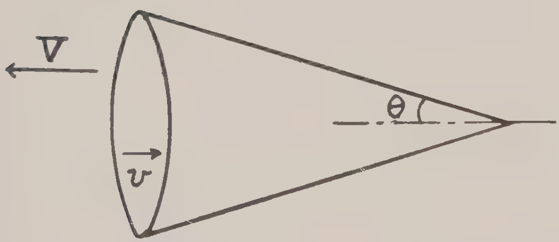


Fig. 1. The model of plankton net; V: towing speed, v: current speed inside the net.

筆者はさき[1]に第一図に示すような円錐形のプランクトンネットを清水中に曳行する場合，ネット入口の流入速度 v とネットの曳航速度Vとの比を濾水率と名付ければ

$$\frac{v}{V}=\frac{\mu}{\sqrt{1+\mu^2}} \quad \cdots\cdots\cdots\cdots\cdots(1)$$

であらわされる事を二三の仮定を設置して，純理論的に導いた。

こゝに　$\mu=\dfrac{c\,\alpha}{\sin\theta}$

c：網目を通過する際の水の流出係数に相当する常数

α：$\dfrac{\text{網目の隙間の総面積}}{\text{網の全表面積}}$

である。本論文に於ては，この様なネットをプランクトンの棲息密度（海水の単位体積中に含まれるプランクトンの個体数）が n である様な海域を曳航した場合に，プランクトンの罹網によつてネットの網目がふさがれ，そのため v の値，従つて濾水率 v/V も次第に減少する状態を理論的に解析したものである。

理 論 的 解 析

式（1）に於て $c/\sin\theta$ はネットの寸法による常数であるから，これをAとおくと

$$\frac{v}{V}=A\frac{\alpha}{\sqrt{1+A^2\alpha^2}} \quad \cdots\cdots\cdots\cdots\cdots(2)$$

ネットの入口の断面積をF，dt 時間にネットに入るプランクトンの量を dN とすれば

$$dN=nFv\,dt$$

これだけのプランクトンによつて網目がふさがれる。そのふさがれ方は，プランクトンの種類や大きさ，

流れの状態等によつて一様ではないが，近似的には此の量 dN に比例するとみとめてよい。

$$\frac{d\alpha}{dt} = -\frac{1}{S}\varepsilon n F v \quad \cdots\cdots\cdots\cdots\cdots(3)$$

こゝにSは網の全表面積，ε は前記の dN に比例すると考えた場合の比例常数である。

（2）式より
$$v = AV\frac{\alpha}{\sqrt{1+A^2\alpha^2}}$$

（3）式に代入して
$$\frac{d\alpha}{dt} = -\frac{1}{S}\varepsilon n FAV\frac{\alpha}{\sqrt{1+A^2\alpha^2}} \quad \cdots\cdots\cdots\cdots\cdots(4)$$

$t=0$ の時，$\alpha=\alpha_0$（α_0 はプランクトンが網目をふさがぬ時の網目の隙間の面積の和と網の全表面積との比）なる条件を入れて，（4）式を積分すれば

$$\frac{\left[\alpha_0\sqrt{\frac{1}{\alpha_0^2}+A^2} - \log\left(\frac{1}{\alpha_0}+\sqrt{\frac{1}{\alpha_0^2}+A^2}\right)\right] - \left[\alpha\sqrt{\frac{1}{\alpha^2}+A^2} - \log\left(\frac{1}{\alpha}+\sqrt{\frac{1}{\alpha^2}+A^2}\right)\right]}{\frac{1}{S}\varepsilon n FAV} = t \quad \cdots\cdots(5)$$

（5）式はVをパラメーターとする α と t との関係を表わすものと考えられる。（5）式から α と t との関係を求め，これを（1）式に代入すれば，v と t との関係が求められるが，式に表わす事は困難であるから各種の常数に適当と思われる値を与えて，V＝100cm/sec と 50cm/sec との場合につき数値計算した結果を図に表わすと第二図の如くなる。

こゝに与えた常数は次の如くである。

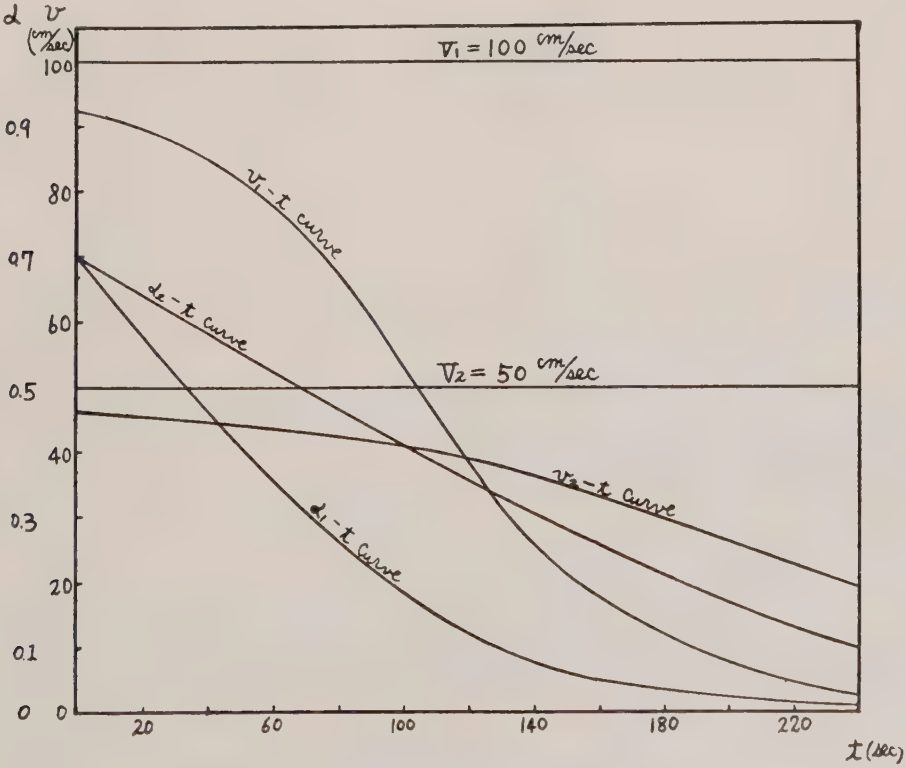


Fig. 2. The relation between v, α and t; α: the ratio of the area of the mesh to the total area of the net, t: towing time.

$$F/S = 0.258$$
$$\varepsilon = 0.5$$
$$n = 0.0005$$
$$A = 3.5$$

第二図について見るに，$V_1=100^{cm}/_{sec}$ の場合のα，v を α_1，v_1，$V_2=50^{cm}/_{sec}$ の場合のそれを α_2，v_2 で表わすと，（5）式より明なる如く，同一の α の値になるまでに要する時間は V_1 の場合に比して V_2 の場合は 2倍を要する。

又（1）式より明なる如く，同一αの点に於ては

$$\frac{v_1}{V_1}=\frac{v_2}{V_2}$$

であるから，t_1 の時の v_1 の値は $2t_1$ の時の v_2 の値の2倍である。従つて次式が成立つ。

$$\int_0^{t_1} v_1\,dt=\int_0^{2t_1} v_2\,dt \qquad \cdots\cdots\cdots\cdots\cdots(6)$$

（6）式の表わす意味は次の如くである。

プランクトンの棲息密度を求めるために用いられている方法は，濾水計又は Flow meter と称する回転式積算流速計を先ず単独で清水中を一定距離L曳航し，その積算回転数をM_0，これをプランクトンネットの中央につけて，海域を同一距離L曳航したる時の読みをM，採捕されたプランクトンの数をNとすれば，海水単位体積内のプランクトンの個数，即密度 n は

$$n=N\frac{M_0}{M_1}\cdot\frac{1}{FL} \qquad \cdots\cdots\cdots\cdots\cdots(7)$$

をもつて表わされるものとされている。積算回転数 M_0 は $V_1 t$ に比例する値であり，M_1 は $\int_0^t v\,dt$ に比例する値であるから

$$n=N\frac{V_1 t}{\int_0^t v_1\,dt}\cdot\frac{1}{FV_1 t}=\frac{N}{F\int_0^t v_1\,dt}=\frac{N}{F\int_0^{2t} v_2\,dt}$$

となつて，速度を半分にすれば時間を2倍，即ち同一距離を曳航する事が必要である事を示している。

結　　論

I）　プランクトンネットでプランクトンを採捕する場合，ネット内部の（ネットの各点で一様と考えている）流速は時間と共に次第に減少するが，その減少の仕方は，曳航速度が大きい程大きい。

II）　所謂濾水率も時間と共に減少する。

III）　一定距離を曳航して採捕したプランクトンの数に濾水計の読みの比（ネットをつけずに清水中を曳航した時の読みと，同一距離をプランクトンネットに取付けて曳航した時の読みの比）を乗じた値より該海域のプランクトン密度を推定するのは妥当な方法である。

IV）　一定時間曳航する場合には速度が早い程濾水率の減少は甚だしい。

尚最後に，本題の解析にあたつて懇切なる御指導並びに論文の御校閲を賜つた当教室小林喜一郎教授，適切なる助言を戴きました本学元田茂教授，安楽正照助手に深甚なる謝意を表します。

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水産動物筋肉中の有機燐酸化合物に関する研究

第4報　鯉肉中の Creatine phosphate に及ぼす貯蔵温度の影響に就て

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Studies on the Organic Phosphates in Muscle of the Aquatic Animals

IV. The effects of storing temperature upon the creatine phosphate content of carp muscle

Tsuneyuki SAITO and Ken-ichi ARAI

Abstract

Post-mortem changes were studied in creatine phosphate content occurring in carp muscle, which had been stored at various temperatures (36°C, 17°C, 4°C, -4°C and in liquid air). The results may be summarized as follows.

(1) When carp muscles were stored at 36°C, 17°C, 4°C, respectively, creatine phosphate was broken down rapidly as the storage temperature became higher.

(2) Stored at -4°C, and after nine hours from start, muscle was solidified by freezing and at the same time creatine phosphate was broken down almost completely.

(3) In liquid air, rapid freezing occurred and muscles were likely to decrease in the amounts of creatine phosphate with the duration of immersion (sec.).

既に著者等は鯉筋肉中の Adenosine polyphosphate の変化と貯蔵温度との関係に関して報告した[1]。その結果によれば，筋肉中の Adenosine diphosphate (ADP) や Adenosine triphosphate (ATP) 等はその貯蔵温度に比例して急速に分解して行くことを知つた。又筋肉を -8°C附近の温度に保ち緩慢凍結を行う時には，凍結現象の開始と同時に ATP，ADP 共に急激に減少し，約5時間経過後の凍結完了と同時に ATP は殆んど失われ，ADP もまたその⅔以上が失われているのを認めた。然しながら鯉筋肉を液体空気中で急速凍結を行つた場合は ATP，ADP の含有量に殆んど変化が認められなかつた。

本研究に於ては鯉筋肉中の Creatine phosphate (CP) と貯蔵温度との関係に就いて追求し，之が既報の ATP，ADP 等の一連の化合物と如何なる関係にあるかを考察して二，三の結果を得たのでこゝに報告する。

実　験　方　法

試料及び測定方法

市販養殖鯉筋肉の処理並びに筋肉中の CP の比色定量法は既に第2報[2] に報告した。
又種々の環境温度に於ける筋肉の保存方法は第3報[1] に於ける場合と同様に取扱つた。
尚鯉の他に試料として使用したドジョウは体長15〜20cmのものを用い，脊肉を左右に2分し一方を常に他方の標準としてその変化を測定した。

実　験　結　果

鯉筋肉を環境温度夫々 36°C, 17°C, 4°C, −4°C に放置し，一定時間経過ごとに CP の量を測定した。そ

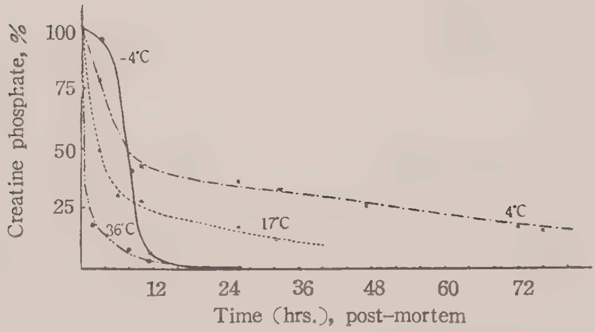


Fig. 1. Changes in creatine phosphate content of carp muscle stored at various temperatures

の結果は第1図に示した。即ち 36°C, 17°C, 4°C の場合では環境温度が低い程 CP の分解速度もまた遅くなる傾向が示されている。然し環境温度が −4°C であるときは筋肉は貯蔵開始後3時間位までは凍結するに至らず透明さも失つていない。この場合には CP 含有量も高い値が保たれている。6時間を経過すれば筋肉は不完全ながらも凍結する。この場合には CP 含有量は急激に減少し，9時間を経過して凍結固化が完全となつた場合には CP 含有量も殆んど失われてしまうことが認められた。この現象は既に第3報[1] でも述べたように，凍結によつて筋肉が収縮を起した結果生ずる現象と解される。

次に著者等は急速凍結によつても同様な現象が起るか否かを検討するために，筋肉を液体空気に浸漬，凍結して同様に CP 含有量を測定しその結果を第1表に示した。1gの筋肉は例外なく15〜17秒で完全凍結す

Table 1. Changes in amounts of creatine phosphate of carp and loach muscles immersed in liquid air

Time immersed in liguid air	Carp γCP/g Muscle								Loach γCP/g Muscle		
	1	2	3	4	5	6	7	8	1	2	3
0 sec.	851	925	869	925	921	725	1097	1274	740	962	1091
3	536		666	851	705	764	1088	1039			
5		509									
15							725	843			
17					425	490					
30								647	740	777	
60	300	407	370	314	392	196	725	725			1239
Diff.	−551	−518	−499	−611	−529	−529	−372	−549	0	−185	+148

るけれどこの凍結完了までの間では液体空気に浸漬した時間に比例して CP が分解される傾向が示された。一方凍結完了後は浸漬時間30〜60秒に至るまで比較的緩慢な減少が認められた。生鮮筋肉の CP 量と60秒凍結後の CP 量との差を変化量として示したがこの値はほぼ500γ/gで大体一定の値を示した。又ドジョウの筋肉に就いて同様の急速凍結を行つた場合に CP の分解が全く認められなかつた。この現象は鯉筋肉の場合と比較して興味が深い。次に鯉とドジョウの筋肉を17°Cに貯蔵した場合の CP 含有量の変化を測定しその結果を第2図に示した。ドジョウの筋肉の CP 含有量は鯉のそれに比較して比較的大きい値を示したが（1.2〜2.2 mg/g）環境温度17°Cに於ける変化は速かであつた。

考　　　察

著者等がさきに Adenosine polyphosphate に就いて得た結果と今回のそれとを併せ考えるならば，鯉筋肉

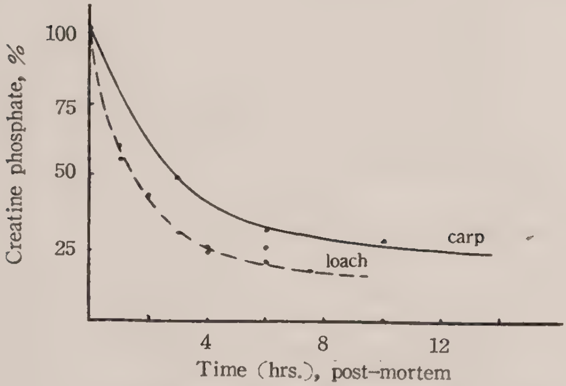


Fig. 2. Changes in creatine phosphate content of carp and loach muscles stored at 17°C

中の高エネルギー燐酸化合物はその環境温度に比例して死後急激に分解し，Bendall[3]の報じたように先ずCPはATP, ADPに比較して一層急激な分解速度を示した。しかもこれら高エネルギー燐酸化合物の筋肉中からの消失時間は大体死後24〜30時間であつて，8〜9°C に於ける鯉の死後硬直到達時間が24〜28時間であるとする安藤等[4]の結果と大略一致している。

又鯉筋肉を−4°C の温度で緩慢凍結を行う際にはCP, ATPは激減し，ADP も相当量消失するのであるが，極低温例えば液体空気で急速に凍結を行うときは CP は可成り分解するがATP及びADPの含有量には殆んど変化は認められない。 著者等が行つたような条件で魚肉を緩慢凍結した際に起る高エネルギー燐酸化合物の変化に関しては，従来殆んど研究は行われていないが，然し液体窒素又は液体プロパン等を使用して，極低温で極短時間処理した場合には之等の物質に殆んど変化が認められないことが報せられている[5]。 然し Caldwell[3] の研究ではドライアイスーアセトンを使用して筋肉を急速凍結した際に，蛙の筋肉では 516γ/g, 亀の筋肉では 15γ/g（湿重量）の割合で筋肉からCPが消失している事実が報告されている。この事実は著者等の認めた鯉とドジョウの場合と対比して或は筋肉組織学的にも興味ある問題ではないかと考えられる。

要　　約

鯉筋肉を種々の環境温度（36°C, 17°C, 4°C, −4°C, 液体空気）に貯蔵してその間に於ける筋肉中の Creatine phosphate の変化を比較定量して次の結果を得た。

(1) 鯉筋肉をそれぞれ 36°C, 17°C, 4°C, に保存した場合には Creatine phosphate の分解の程度は温度の上昇にともなつて大となる。

(2) 鯉筋肉を−4°C の冷蔵庫に保存すれば，貯蔵開始後約9時間で筋肉は凍結固化する。凍結現象と同時に Creatine phosphate は殆んど完全に分解する。

(3) 鯉筋肉を液体空気に浸漬すれば， 急速に凍結が行われるが， 1分間以内の浸漬結果では浸漬時間に比例して Creatine phosphate は減少の傾向を示す。然しドジョウの筋肉の場合は顕著な変化は認められない。

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結晶鯨インシュリンに関する研究

第2報 アミノ酸組成

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Studies on Crystalline Whale Insulin

II. Amino acid composition

Tsuneyuki SAITÔ, Yoshio ISHIHARA, Yasuzo ITÔ and Masahiko FUJINO

Abstract

As reported in the previous paper, the authors were able to prepare sufficient quantity of crystalline whale insulin for the purpose, and therefore, the complete analysis of the protein was undertaken. The amino acid composition of hydrolysates of crystalline whale insulin has been determined by chromatography on columns of Dowex 50-X8. The corrected analytical values yield integral numbers of residues for most of the amino acids and account for 97.64 per cent of the nitrogen and 94.51 per cent of the weight of whale insulin.

The analyses indicate the following 48 amino acid residues in the whale insulin molecule (min. mol. wt. 5719 C_{253} H_{375} N_{65} O_{75} S_6): Asp_3 Glu_7 Gly_4 Ala_3 Val_4 Leu_5 $Ileu_2$ Ser_3 Thr_2 CyS_3 Pro_1 Phe_4 Tyr_3 His_2 Lys_1 Arg_1 $(-CONH_2)_6$.

緒言

今迄に牛・豚・羊並に各種硬骨魚の結晶インシュリンが単離され，之等の中牛・豚・羊インシュリンに就てはアミノ酸組成が定量せられて，元素組成・結晶形・生物活性・向流分配，並にアナフィラキシー反応・補体結合反応等の免疫学的見地からも種属特異性が認められないのに拘らず[1,2]，Thr, Ser, Gly, Ala, Val, Ileu 等のアミノ酸含量に差異を示す事が明かにされている。[3-6]

先にインシュリンの一般製法を各種比較し亜鉛塩添加の柴田法[8]の優秀性を確認し，更に結晶鯨インシュリンの単離を報告[7]したが，ここにその構成アミノ酸の完全分析を行つた結果，前記インシュリン同様に Met, Try, CySH を欠くも Asp, Glu, Pro, CyS, His, Lys, Arg 以外の含量に若干の種属差異を認めたので報告する。

実験の部

試料——前報[7]のイワシ鯨(*Balaenoptera borearis Lesson*)の結晶インシュリンを2回再結を繰返した後アセトン洗滌。真空デシケーター内で乾燥供試した。

加水分解——結晶インシュリンを10倍量の6N HCl と共に封管中110°Cに24時間及72時間加熱して加水分解した後減圧下過剰の塩酸除去後常法により Buffer で pH 調節定容後一定量を供試。

アミノ酸分析—— Moore & Stein 法[9]に従つて行い別に CyS の定量を Schram, Moore & Bigwood 法[10]，Tyr の定量は紫外部吸収法を併用した。加水分解に於ける個々のアミノ酸の分解に対する補正は24時間及72時間両水解物の定量数値から外挿法に従つて行つた。

実験結果並に考察

実験結果は Fig. 1 及 Tab. 1, 2 に示す如くである。

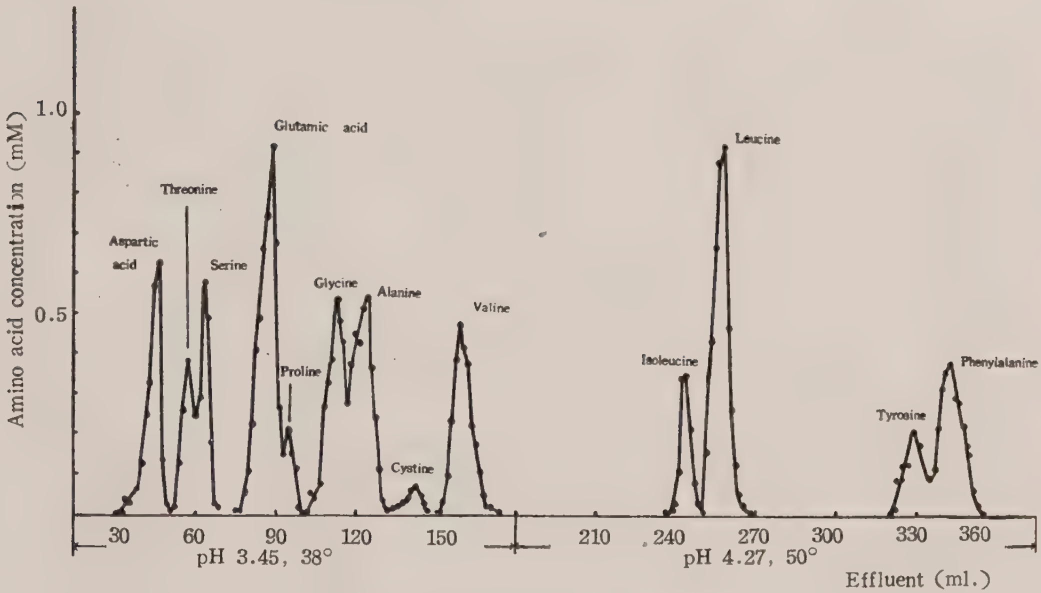


Fig. 1-a. Typical effluent curves for 24-hour hydrolysate of whale insulin (100cm. column of Dowex 50−X8)

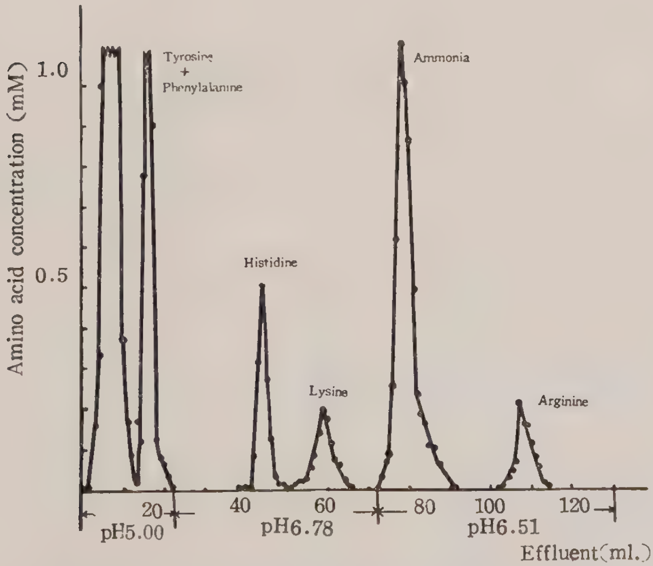


Fig. 1-b. Typical effluent curves for 24-hour hydrolysate of whale insulin (15cm. column of Dowex 50−X8)

定性的に見る時は Met, Try, CySH を欠き（別に Try に就ては Cole の反応，紫外部吸収の定性試験何れも negative, CySH に就ては Nitroprusside 反応等で negative）之迄のインシュリンと全く同一である。
先にペーパークロマトグラフイーで α− aminobutyric acid が認められたが今回は全く検出されず二次的分

Table 1. The Amino Acid Composition of Whale Insulin

Amino Acid	Amino acid per 100 g. protein	Amino acid residue per 100 g.protein	N as % of total N	No. of residues per mole of insulin
Aspartic acid	6.74 ± 0.09 g.	5.84 ± 0.08 g.	4.52 ± 0.06	3.04 ± 0.04
Threonine	3.34 ± 0.14	2.84 ± 0.12	2.49 ± 0.10	1.68 ± 0.07
Serine	4.97 ± 0.02	4.11 ± 0.02	4.22 ± 0.02	2.84 ± 0.01
Glutamic acid	16.29 ± 0.06	14.95 ± 0.05	10.32 ± 0.04	6.65 ± 0.02
Proline	1.46	1.23	1.13	0.76
Glycine	5.08 ± 0.02	3.86 ± 0.02	6.04 ± 0.02	4.05 ± 0.02
Alanine	5.03 ± 0.02	4.01 ± 0.02	5.04 ± 0.02	3.39 ± 0.01
Cystine *	11.09	9.43	8.23	2.77
Valine	7.56 ± 0.19	6.40 ± 0.16	5.76 ± 0.14	3.88 ± 0.09
Isoleucine	5.08 ± 0.20	4.38 ± 0.17	3.46 ± 0.14	2.33 ± 0.09
Leucine	11.55 ± 0.08	9.97 ± 0.07	7.87 ± 0.05	5.29 ± 0.03
Tyrosine	8.62 ± 0.04	7.76 ± 0.03	4.25 ± 0.02	2.86 ± 0.01
Phenylalanine	10.78 ± 0.11	9.59 ± 0.08	5.83 ± 0.06	3.92 ± 0.04
Histidine	5.10 ± 0.23	4.51 ± 0.20	8.80 ± 0.43	1.97 ± 0.09
Lysine	2.62 ± 0.30	2.30 ± 0.26	3.20 ± 0.37	1.08 ± 0.12
Arginine	3.72 ± 0.16	3.33 ± 0.14	7.62 ± 0.33	1.28 ± 0.05
Ammonia	....	....	8.86 ± 0.46	5.96 ± 0.31
Total	109.03	94.51	97.64	

* Measured as cysteic acid but calculated as cystine.

Table 2. Effect of Prolonged Hydrolysis Time on the Amino Acid Composition of the Whale Insulin

Amino acid	Number of residues		
	24 hr.	72 hr.	Average or extrapolated values
Aspartic acid	2.82	2.45	3.04 *
Threoine	1.63	1.52	1.68 *
Serine	2.59	2.09	2.84 *
Glutamic acid	6.13	6.54	6.65 **
Proline	0.74	0.71	0.76 *
Glycine	3.97	4.12	4.05
Alanine	3.39	3.39	3.39
Valine	3.34	3.77	3.88 **
Isoleucine	1.61	2.18	2.33 **
Leucine	5.28	5.31	5.29
Tyrosine	2.47	1.67	2.86 *
Phenylalanine	3.14	3.80	3.92 **

* Extrapolated to zero time of hydrolysis.
** Averages of the recoveries after 72- & 96-hour hydrolysates.

解生成物であつたものと考える。

定量的には之迄の牛・豚・羊三種インシュリンと比較して種属差が認められる。

アミノ酸含量の定量分析に於ては蛋白の水解時に於るアミノ酸の破壊（或いは逆に水解不充分の為のpeptide残存）に対する補正が難しい問題であるが水解時間を延長しての結果と外挿法で処理した。Tyrは紫外部吸収による定量結果と一致した。CySは別にperformic acidで酸化した蛋白内のCysteic acidをSchram等[10]のDowex 2を用ひてのこの定量法を併用した。水解時間を異にした場合の個々の分解程度はTab. 2に示す如くであるが，Harfenistの結果と傾向を同じくしているがAspのみが逆である。之はHirs, Stein & Moore等[11]の場合が我々と同じ傾向を示している。N回収率及アミノ酸残基の含有率共に良好であるので以上の処理で充分と考える。

鯨インシュリン1モル当りの各アミノ酸残基数を見る為にN-末端のGly及Pheの定量結果よりMmin. 平均5900が得られるので（之は次回に報告の予定，尚アミノ酸残基からのbest Mmin. も約6,000である）今Mmin. 6,000とした時の結果をHarfenistの測定値と比較するとTab. 3の如くである。（インシュリン

Table 3. Amino Acid Compositions of Insulins from Different Sources

Amino acid	Number of residues				
	Whale	Beef-A[5]	Beef-B[5]	Pork[5]	Sheep[5]
Aspartic acid	3	3	3	3	3
Threonine	2	1	1	2	1
Serine	3	3	3	3	2
Glutamic acid	7	7	7	7	7
Proline	1	1	1	1	1
Glycine	4	4	4	4	5
Alanine	3	3	3	2	3
Cystine	3	3	3	3	3
Valine	4	5	5	4	5
Isoleucine	2	1	1	2	1
Leucine	5	6	6	6	6
Tyrosine	3	4	4	4	4
Phenylalanine	4	3	3	3	3
Histidine	2	2	2	2	2
Lysine	1	1	1	1	1
Arginine	1	1	1	1	1
Ammonia	6	6	5	6	6

のアミノ酸含量に関しては前記Sanger, Harfenistの他に最近もGrassmann等[12]の報告がありIleu及Valの含量に多少の問題がある様であるがここではHarfenistの結果を取る）。

Tab. 3により鯨・牛・豚・羊の4種インシュリンのアミノ酸含量を比較すると――4種インシュリン共同一含量のアミノ酸：Asp, Glu, Pro, CyS, His, Lys, Arg及アミド基。モル当りの残基数も4種同一である。鯨のみが特異的に異るもの：Leu, Tyr, Pheに対し羊ではGly，豚ではAlaが特異的に量を異にして存在するアミノ酸である。

この他鯨インシュリンはThrが牛より1分子多く豚と同一，Valは牛より1分子少く豚と同一，Ileuが牛より1分子多く豚と同一で豚インシュリンに最も近い類似性を示す。但し豚よりAlaとpheを各1分子多くLeuとTyrを各1分子少く含有している。この様に4者共アミノ酸残基数は同一で且Mmin. も極めて酷似しているも構成アミノ酸含量を相互に若干異にしている。

インシュリンのホルモン活性と構造との問題に於て，ACTH, Oxytocin, Vasopressinの如き小分子の

peptide fragment を酸，アルカリ，各種蛋白酵素による水解で得ようと Freudenberg[13)–16)]，Butler[17)18)]，Phillips[19] 及 Harris[20] 等多数の研究者が努力し来つたが，S-S 結合[13] phenol 性 OH[21]，若干の遊離 carboxyl 基[13] 等が活性に関係ある事，C-末端の Asp (NH_2)[20] が或程度関係する事が判明したに止まる。同様の報告[22] が昨年も出されたが矢張り negative work であつた。但し Rockefeller 研究所の Merrifield & Woolley[23] の Strepogenin 活性の pentapeptide (Ser-His-Leu-Val-Glu——先に Sanger も取出している) を証明した事は注目に値する。近い将来新しい発展が期待される。4種インシュリンを比較する時若干の種属差が見られるが生物活性その他殆ど同一である事は之らの種属差を示すアミノ酸のインシュリンの性質に与える影響が小なる事を示し，従つてホルモン活性は之等アミノ酸とは関係が薄いものと考える。

要　　約

結晶鯨インシュリンのアミノ酸組成を Moore & Stein の Dowex 50 のイオン交換クロマトグラフ法により定量し豚・牛・羊等陸上動物のインシュリンとの種属特異性を見た。

(本実験遂行に当り本学村田研究室の各位及び久保周一郎講師に多大の御援助を頂いた。厚く謝意を表す。)

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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

III. The Crystallizing State of Chemically Synthesized Crystals at Various Temperatures

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In the previous paper[1], the authors have studied the solubility of chemically synthesized crystals of $MgNH_4PO_4 \cdot 6H_2O$, it was shown that the maximum solubility was at 50°C, and that the solubility became less below or above a boundary of 50°C. From this fact, the crystals are considered to be easily crystallized out at below or above 50°C. However, the crystallizing states at lower or higher temperatures than 50°C are presumed to be different.

In order to ascertain the temperature at which visible crystals generate in commerical products, the authors have observed the size and the shape of the crystals when the component ions are combined at various temperatures and then cooled to various temperatures.

1. In the case when the combining temperature and leaving temperature are the same

The component ions are combined at respective definite temperatures; the conditions of the crystals which generate and are left at the same temperature were observed.

(1) *Experimental material*

One-one hundredth Mol magnesia mixture solution which was prepared according to the method described in the foot note of this page, and 0.01 Mol sodium phosphate were made separatedly and were used for the experiment.

(2) *Experimental apparatus*

The apparatus used in this experiment was the same as the apparatus shown in the previous paper[1].

(3) *Experimental method*

Each 20 cc of 0.01 Mol magnesia mixture solution and 0.01 Mol sodium phosphate

Foot note: Magnesia mixture solution was prepared by mixing 55 g of anhydrous magnesium sulfate with 70 g of ammonium chloride in 400 cc of dist. water, to which 500 cc of 5 % ammonia water was added, and finally was added to make the total volume 1,000 cc.

This solution was diluted with water to make 0.01 Mol on the basis of the quantity of magnesium sulfate in the solution.

solution was combined in large test tubes (160 cc volume) and heated at respective temperatures from 100°C to O°C for 30 minutes. The shape and size of the crystals generated were observed under a microscope.

(4) *Experimental result*

Photographs of the shapes of the crystals are shown as Fig. 1.

As seen in Fig. 1, the shapes of the crystals are different according to the combining temperatures. As to the boundary of 50°C, the shapes of the crystals generated above 50°C are amorphous, but the shapes below 50°C are beautiful crystals having regular faces. When those crystals are observed under a polarizing microscope, the former did not show polarized light, but the latter did. The difference of the shapes of the crystals generated above or below 50°C is considered to be connected with the maximum solubility of the crystal at 50°C as the boundary.

2. In the case of combining of the component ions at about 100°C and the leaving temperature being allowed to descend to the respective definite temperatures

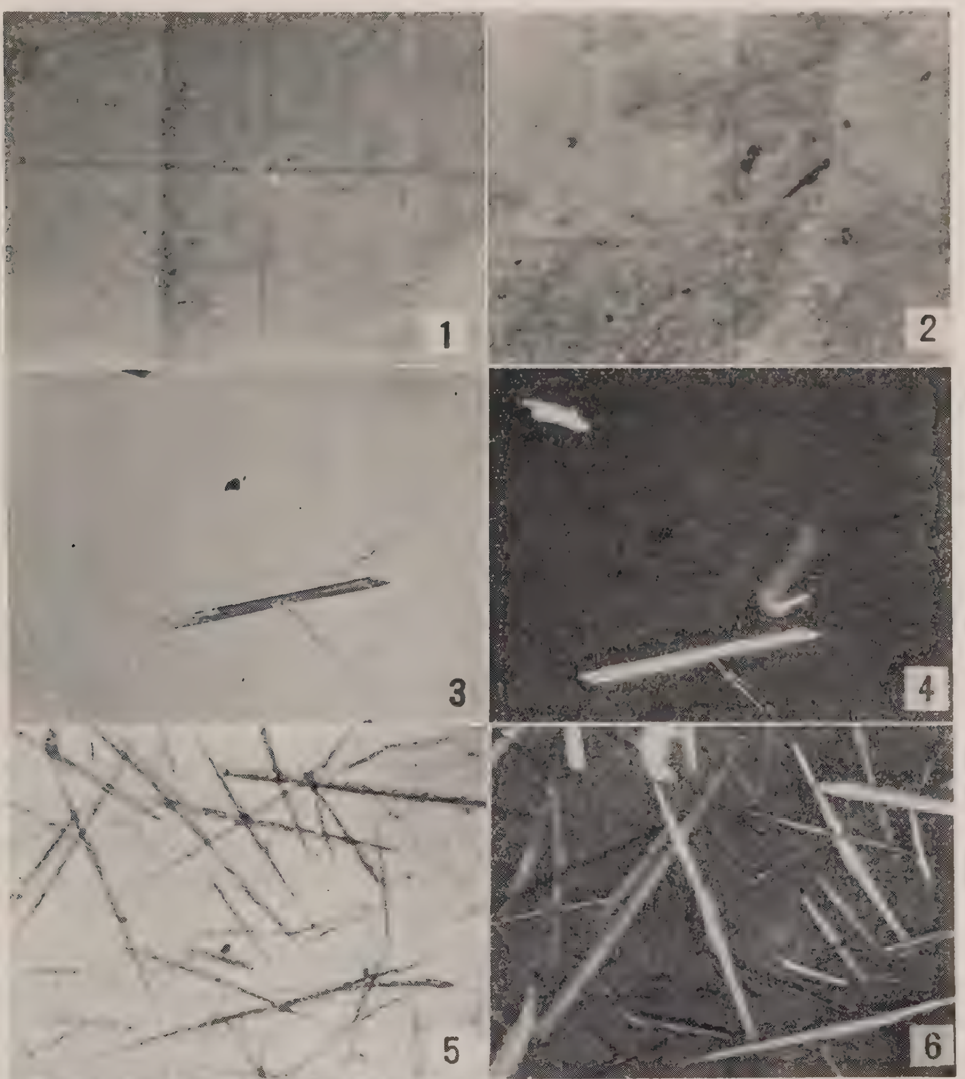
Usually when canned foods are processed above 100°C, the component ions of the struvite in the content will combine and the size and the shape of the crystals generated in the canned foods are considered to change in accordance with the cooling conditions under which the temperature of the center of the canned food is changing.

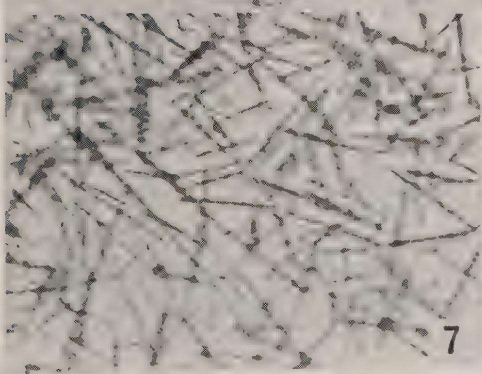
Let it be supposed in the processing of the canned foods, that magnesia mixture solution and sodium phosphate solution were combined at comparatively higher temperature (at about 100°C) and the mixture was cooled to the respective definite temperature by rapid or slow coolings. The authors have observed the shape and size of the crystals generated.

(1) *Experimental method*

A heating bath shown as Fig. 1 in the previous paper[1] was kept at 98° ~ 100°C. Each 20 cc of 0.01 Mol magnesia mixture solution and 0.01 Mol sodium phosphate were poured into a large test tube which was kept at 98°~100°C. Then cold water was poured into the heating bath in order to change the velocity cooling (rapid cooling or slow cooling). The changes of shape and the size of the crystals are influenced by cooling temperatures and cooling velocities were observed.

"Rapid cooling" means the cooling velocity occurring when the canned foods are cooled in cooling tank water after the processing. "Slow cooling" is the velocity in cooling by electric fun. These cooling velocities are regulated in accordance with the practical cooling of canned foods. Moreover, "very rapid cooling" means to cool the test tube rapidly in running city water after combination of both component ions without





1. Crystals which were formed at 60° ~ 70°C
2. Crystals which were formed at 50°C
3. Crystals which were formed at 40°C
4. Polarized photograph of crystals which were formed at 40°C
5. Crystals which were formed at 30°C
6. Polarized photograph of crystals which were formed at 30°C
7. Crystals which were formed at 20°C

Fig. 1. Shapes and sizes of crystals generated at 100°~0°C and left at the same temperatures

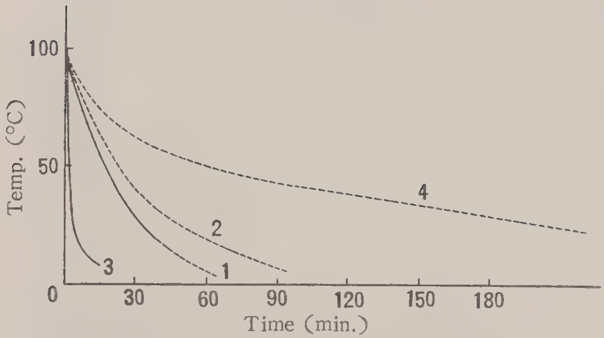


Fig. 2. Cooling velocities of the combined component ions
1: Rapid cooling 2: Slow cooling
3: Very rapid cooling 4: Very slow cooling

accordance with the cooling velocity of processed cans. "Very slow cooling" is to cool the test tube slowly by leaving it in the air.

(2) *Experimental result*

The results obtained are shown in Table 1.

As seen in Table 1, when the component ions were combined at 98° ~ 100°C and were cooled to the respective definite temperatures, the size and the number of the crystals generated are different according to the cooling velocities. When the mixture of the component ions was cooled by very slow cooling, a few amorphous crystals and a few large crystals having regular faces were found. When the mixture was cooled by rapid cooling or very rapid cooling, many amorphous crystals and many small crystals having regular faces were found. That is to say, when the mixture is rapidly cooled, many invisible small crystals having regular faces are found.

It is important that when the component ions are combined at comparatively higher temperatures and are cooled, the size of the crystals formed at 50° ~ 30°C is larger than those at below 20°C; the number of the crystals having regular faces is less than those at other temperatures. The authors have called those limits of the temperature, 50° ~ 30°C, **"Zone of the formation of large crystals".**

In the case of rapid cooling, when the component ions were combined at 98° ~ 100°C and were cooled to below 20°C, small invisible crystals having regular faces were found. The size of the crystals generated by rapid cooling is smaller than the size of those by slow cooling, but the number is the contrary. That is to say, in the slow cooling, when the component ions were combined at 98° ~ 100°C and then were cooled to any temperatures, a few pretty large visible crystals were found. In short, when the crystals generated have rapid temperature changes, the change of supersaturation of the solution becomes large. Therefore, the crystallization become rapid, the growth of the crystals generated becomes small, then it is reasonable to expect to find many small crystals. However, on the contrary, in the case of slow cooling, the change of supersaturation of the solution becomes small; then the crystallization becomes slow, therefore a few crystals generated becomes nuclei. These nuclei grow to large size, and a few large shaped crystals may be expected to result.

Table 1. The shapes and sizes of crystals which were generated at 98°∼100°C and the leaving temperature were allowed to descend to the respective temperatures

Cooling method	State of the crystals \ Temperature change	100°→70°C		100°→50°C		100°→40°C		100°→30°C		100°→20°C		100°→10°C	
		Size (μ)	Number (per 0.1 mm^3)	Size (μ)	Number (per 0.1 mm^3)	Size (μ)	Number (per 0.1 mm^3)	Size (μ)	Number (per 0.1 mm^3)	Size (μ)	Number (per 0.1 mm^3)	Size (μ)	Number (per 0.1 mm^3)
Rapid cooling	Amorphous	1 × 1	many	1 × 1	many	1 × 1	many	1 × 1	many	1 × 1	few	1 × 1	few
	Crystals having regular faces	14×7 ∼40×14	15	16×8 ∼150×25	20	16×8 ∼149×30	25	16×8 ∼150×33	24	13×7 ∼16×57	65	13×3 ∼57×8	73
Slow Cooling	Amorphous	1 × 1	many	1 × 1	many	1 × 1	many	1 × 1	many	1 × 1	few	1 × 1	few
	Crystals having regular faces	16×8 ∼50×3	13	33×8 ∼82×16	15	49×8 ∼82×16	15	41×16 ∼150×49	13	49×16 ∼120×49	20	49×16 ∼310×49	22
Very rapid Cooling	Amorphous	1 × 1	many	1 × 1	many	1 × 1	many	1 × 1	many	1 × 1	few	1 × 1	few
	Crystals having regular faces	8×3 ∼33×16	7.5	8×3 ∼33×16	30	33×16 ∼40×16	20	16×3 ∼33×8	25	8×3 ∼25×8	45	8×3 ∼25×8	55
Very slow Cooling	Amorphous	1 × 1	few	1 × 1	few	1 × 1	few	1 × 1	few	1 × 1	few	1 × 1	few
	Crystals having regular faces	8×8 ∼82×25	9	49×16 ∼110×33	7	8×8 ∼49×8	20	98×16 ∼180×16	6	49×16 ∼180×33	13	49×16 ∼450×33	10

Summary

In the crystallization of $MgNH_4PO_4 \cdot 6H_2O$, when the component ions are combined above 50°C and left, amorphous crystals generate. But when the combination is done below 50°C and when the generated crystals are left undisturbed, crystals having regular faces are found. On the contrary, in the case of combination at 98° ~ 100°C, when the leaving temperature is 90°C, amorphous crystals, and when the leaving temperature is 70°C, a few crystals having regular faces as well as many amorphous crystals are found.

Visible crystals having regular faces are found only when the combination is done at 98° ~ 100°C and temperature is allowed to descend to below 50°C. The crystals formed at 50° ~ 30°C are larger than those at other temperatures. The authors have called those limits of the temperature, the **"Zone of the formation of large crystals".** The size of the crystals generated by rapid cooling is smaller than that by slow cooling; the former are more abundant than the latter. The more rapid the cooling velocity, the smaller are the crystals which generate. This fact suggests a method for making the crystals of struvite in the canned foods invisibly small.

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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

IV. Formation of the Crystal of $MgNH_4PO_4 \cdot 6H_2O$ and its Minimum Ion Concentration

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In the previous paper, the authors[1] have pointed out that the minimum concentrations of the components of the crystal will be necessary for its formation. In this paper, the authors have studied the minimum ion concentration needed for the formation of $MgNH_4PO_4 \cdot 6H_2O$.

Popoff and Neuman[2] have microscopically observed how the rate of addition, and the temperature at which addition should be made, etc., influence the shape and size of the generated crystals, in mixing of barium chloride with potassium sulfate. The authors have also observed under a microscope the size and shape of the crystal of $MgNH_4PO_4 \cdot 6H_2O$ in combination of the components of the struvite from the canned foods at various temperatures and have discovered the minimum concentration of the components necessary for the formation of the crystal.

Experimental method

Solutions A and B were prepared for the experiment. Solution A is magnesia mixture which was prepared as well as in the previous paper[3].

Solution A was diluted with water to make from 0.4 Mol to 0.0001 Mol on the basis of the quantity of magnesium sulfate in the solutions. Solution B is sodium phosphate solution, from which various concentrations of the solution were prepared from 0.4 Mol to 0.005 Mol. Each 10 cc of A and B solutions of the same concentration were mixed separatedly in Petri–dishes at room temperature (19° ~ 20°C) and 40°C. There were two mixing rates: instant and 2 minutes. Then the conditions of crystals formed by the mixing were compared in reference to the differences of the concentration of the solution, temperatures and mixing rates. When the mixing was completed, the solution in the dish was stirred by moving the dish by hand. Each 0.05 cc of the mixed solution was taken onto the slide glass by pipette, and the size of the formed crystals was measured under the microscope by micrometer. The number of the formed crystals was also measured on the counting chamber of Thoma's haemacytometer by counting three divisions having definite area.

Experimental results

The results obtained are shown in Tables 1 and 2.

Table 1. In the case of mixing A and B

Concentration of the solution	0.4 Mol		0.2 Mol		0.1 Mol		0.05 Mol	
Adding time	Instant	2 minutes	Instant	2 minutes	Instant	2 minutes	Instant	2 minutes
Number of crystals (per 1 cc)	56×10^4	60×10^4	24×10^4	24×10^4	56×10^4	60×10^4	24×10^4	8×10^4
Size of crystals (μ)	18×1.3	63×2.5	14×2.5	25×2.5	120×2.5	126×2.5	92×1.5	330×1.3
State of crystals	A B	A B	A	A	A	A′	B	A′B

Note: Signs which are described in this table show the states of the formed
A=arborescent B=broad blanches C=columnar crystal D=columnar crystal

Table 2. In the case of mixing A and B solutions at temperature of 40°C

Concentration of the solution	0.4 Mol	0.2 Mol	0.1 Mol	0.05 Mol	0.02 Mol	0.01 Mol	0.005 Mol
Adding time	Instant	Instant	Instant	Instant	Instant	Instant	Instant
Number of crystals (per 1 cc)	200×10^4	160×10^4	160×10^4	16×10^4	10×10^4	—	80×10^4
Size of crystals (μ)	26×2.6	26×4.0	50×2.6	50×3.1	82×1.2	120×1.2	8.2×1.3
State of crystals	C B	C B	C B	C	C	D	D

Note: Signs which are described in this table show the states of the formed crystals as follows.
B=broad blanches C=columnar crystal
D=columnar crystal separated

(1) The difference of conditions of the crystal formed by different concentrations of the solutions

As seen in Table 1 and 2, in the range of 0.02 Mol and 0.0025 Mol, the more diluted the solution is, the more regular is the form of the crystals. Above 0.05 Mol, the form of the crystal was irregular, and the crystals of needle shape gathered into arborescent colonies like frost that is found on the window glass on cold days.

The more diluted the solution is, the less is the number of crystals. Visible sized crystals are formed by mixing of A and B solutions having each above 0.01 Mol in which case the mixture contains 0.0122 % of Mg.

solutions at room temperature (19°~20°C)

0.02 Mol		0.01 Mol		0.005 Mol	0.0025 Mol	0.001 Mol	0.0005 Mol	0.0001 Mol
Instant	2 minutes	Instant	2 minutes	Instant	Instant	Instant	Instant	Instant
32×10^4	10×10^4	24×10^4	14×10^4	10×10^4	8×10^4	12×10^4	16×10^4	4×10^4
54×1.3	239×1.3	59×0.9	63×1.0	40×2.2	24×7.6	37×2.7	57×3.8	1.3×1.0
C B	D	D	D	C E	C E	C E	E	E

crystals as follows.
separated E=amorphous A′=rather large arborescent B′=rather large broad blanches

(2) Difference of conditions of the crystals obtained by different mixing rate

When each 10 cc of A solution is poured into each sample of B solution instantly or in 2 minutes, the size and form of the crystals varied by the mixing rate (mixing time) as shown in Table 1.

The shapes of the crystals in the instantly mixed solution are irregular and the sizes are small. But the forms of the crystals in the slowly (2 minutes) mixed solution are regular and the sizes are large. The difference of the influence upon the form and size of the crystals by the different rate of the mixing remarkably appeared in the concentration of each solution A and B when their make-up was above 0.01 Mol.

(3) Difference of conditions of the crystals obtained under different temperatures

As seen in Tables 1 and 2, the size and shape of crystals formed at room temperature (19° ~ 20°C) or at 40°C are different. The form of the crystals formed at 40°C seems to be more regular and more compact than the crystals formed at room temperature, even from the same concentration of both solutions. The former showed no arborescent crystals unlike the latter. And the crystals formed at room temperature are inseparably connected with each other. They give an impression of çoarseness.

Summary

The authors have studied the factors influencing the size and shape of the crystals of $MgNH_4PO_4 \cdot 6H_2O$, when the chemical components of the crystal, magnesia mixture solution (magnesium sulfate and ammonia water), (A solution) and sodium phosphate solution, (B solution) are mixed in the same concentration in the range between 0.0001

Mol and 0.4 Mol at different temperatures (room temperature and 40°C) and at different mixing rates (instantly and during 2 minutes).

The visible crystals are formed by mixing of the two solutions above 0.01 Mol. The amount of Mg in the mixed solution yielding the visible crystals was 0.0122 %. The crystals which were formed by mixing of the two solutions above 0.05 Mol, have irregular forms which look like arborescent colonies of needle crystals. The crystals which were formed by mixing of the two solutions below 0.01 Mol are invisible and microscopic.

The crystals which were formed by instant mixing of the solutions have more irregular shape and are smaller than those crystals which were formed in two minutes mixture. The mixing rate exerts scarcely any influence upon the difference of the size and form of the crystals in the case of diluted solutions below 0.01 Mol.

The shapes of the crystals which are formed at 40°C are more regular than those at room temperature (19° ~ 20°C).

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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

V. The Growth of the Crystals of $MgNH_4PO_4 \cdot 6H_2O$ in Glass Vessel

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If the respective chemical constituents of the struvite are existing in amounts over a definite (minimum) quantity in canned sea foods, the constituents will combine with each other in the juice as a result of heating (sterilization), and some nuclear crystal will be formed. The nuclear crystals[1] will grow to visible size, if surrounding factors, *e. g.*, temperature, the concentration of the solution, pH, etc. are kept in the suitable condition.

As the growth of the struvite in the canned foods will be influenced by various intricate factors, it is difficult to clear up the mechanism of the formation. Therefore, the authors have at first tried to find out the formation of $MgNH_4PO_4 \cdot 6H_2O$ in the true solution in glass vessel.

1. In the case of storing of the mixing solution without heating

(1) *Experimental method*

After each solution[2] of A and B was mixed, the mixture was kept in a thermostat of 37° ± 1°C. The size and form of the crystals which formed were observed on every other day. For the observation, 0.05 cc of the solution was taken onto a slide glass by glass tube, and size and shape of the crystals were observed under a microscope three times for each observation to obtain the averaged values.

(2) *Experimental result*

Results obtained are shown in Table 1.

As seen in Table 1, it is evident that there is a relation between the change of the form and size of the crystals at the time of the storing of the mixed solution and the rate of mixing the solutions.

The shape of the crystals formed in the slowly (within two minutes) mixed solution did not change even if the crystals were stored for a long time; on the other hand, the shape of the crystals formed in the instantly mixed solution changed during the storing.

The size of the crystals formed in mixtures at both rates increased with aging. Further, as seen in Table 1, the conditions of the aging and the growth of the crystals vary by the concentrations of the solution. The higher the concentration of both solution, the greater is the number of floating crystals on the surface of the solution. The crystals formed from the mixed solution of 0.01 Mol (0.0122 % of Mg) and 0.02 Mol

(0.0224 % of Mg) appeared at the bottom of the Petri-dish after 80 ~ 120 hrs.

The amount of Mg in the mixed solution of 0.01 Mol ~ 0.02 Mol corresponds to the minimum amount of Mg in the pickle of canned crab. The fact that there are many struvites in the bottom of the canned crab which is covered with pickle is perhaps due to the same causes.

Next, it was undertaken to observe the form of the crystals grown during the storing. The arborescent crystals formed at the initial period of the storing collapse gradually and divide into small crystals during long storing time. As time goes on, the divided crystals gradually become shaped ones. This may be considered to occur as follows: the phenomenon of dissolution will occur partially on the surfaces of the divided crystals and the partly dissolved crystals will be recrystallized to form the regular shape.

2. The growth of the crystals formed in the mixed solution during the storing after heating

(1) Experimental method

Each 5 cc of solutions[2] A and B of concentrations 0.2~ 0.005 Mol were mixed with each other. Each mixed solution was heated at 109.9°C for 2 hrs., like the sterilization of canned crab and cooled gradually at room temperature, then stored for 25 days. The visible crystals which formed were observed.

(2) Experimental result

Results obtained are shown in Table 2.

Table 2. Change in the crystal during the aging after the heating of the mixture of the component ion

Concentration of the reagents (Mol)		0.2	0.1	0.05	0.033	0.025	0.02	0.01	0.005
The ratio of each components	Mg (%)	0.153	0.075	0.038	0.025	0.018	0.015	0.0075	0.0038
	PO_4 (%)	0.207	0.103	0.052	0.035	0.026	0.021	0.010	0.0052
	NH_3 (%)	0.093	0.047	0.023	0.016	0.012	0.0093	0.0047	0.0023
Visible crystals		+	+	+	+	+	+ / −	− / +	−
Microcrystals		+	+	+	+	+	+	+	+
Form of the crystals		A E	1000~ 2000× 3000 D′	3000~ 5000× 8000 D′	500~ 1000× 1000 C		126× 63 D E	60×20 D E	20×6 C

Note: Signs which are described in this table show the states of the formed crystals as follows.

A=arborescent C=columnar crystal D=columnar crystal separated
D′=rather large columnar crystal E=amorphous

As seen in Table 2, the minimum amount of Mg, by which the visible crystals can be formed, is above 0.015 % Mg, *i.e.*, 0.02 Mol of each solution. This substantiates positively the results reported in previous paper III[2]. In the more diluted solutions (0.0038 % of Mg), a few microscopical crystals whose sizes are about 20 × 60 μ are formed.

As stated in previous paper II[3], if Mg exists to an amount more than 0.0025 % in the mixed solution, the microscopical crystals are observed to begin to appear at about 25°C.

In both cases of the mixed solution heated or unheated, the size and number of the crystals formed differ from the concentration of the mixed solution rather than from heating. The higher the concentration is, the smaller in size and the more in number are the crystals. In the higher concentration of the mixed solution, the crystallization is more rapid, therefore the crystals are many and floating.

In the solution of the concentrations of 0.02 Mol ~ 0.01 Mol, the crystals attached to the side wall of Petri-dish generated after 80 ~ 120 hrs. In those solution, each chemical constituents exist saturatedly according to temperatures. Therefore, the constituents combine with each other and some solid crystallize out by a medium of a part of side wall of the dish as nucleus, and then the nuclear solid will grow gradually.

The comparatively more regular form of the crystals will generate and grow from the concentration of 0.02 ~ 0.01 Mol at about 37°C.

From the agreement of the amount of Mg employed in the above described experiment with the amount of Mg in the pickle of canned crab, and the storing temperature after the processing being 30° ~ 40°C in holds of floating canneries, it may be stated that nuclear crystals attaching to the inside wall of can container or to the surface of the crab meat generate and grow to become struvites. When the conditions of the formation are suitable, the struvites grow much larger. During the aging of the crystals formation, the crystals having coarse surfaces dissolve once, because of the difference of the solubilities at different temperatures and then the regular surface of the crystals will be completed by the mechanisms as above stated.

3. The influence of temperature upon the growth of the crystals which have been formed by different cooling velocities and were left at different temperatures

In the previous paper[2], the shape and size of the crystal were observed to be different according to the velocity of cooling. Here the changes of the growth of the crystals which have been formed by different velocities of cooling were observed under different storage temperatures.

(1) *Experimental method*

In order to experiment on the formation of the crystals under similar conditions

to those pertaining in the canned food juice, 0.05 Mol magnesia mixture solution and 0.05 Mol sodium phosphate solution containing 1% pepton were prepared. Each 5 cc of the two solutions were mixed in 4 separate large test tubes. In each mixture, pepton was added to 0.5 %. The 4 test tubes containing the mixture solutions were heated at 5 *lbs* (108.4°C) for 90 minutes. Each two test tubes were cooled by rapid or slow cooling as previously defined. After cooling, one of the two test tubes was kept at room temperature (15° ~ 20°C) and the other was kept at 37°C, respectively. At definite intervals the shape and size of the crystals which were formed in the respective 4 test tubes were observed under microscope.

(2) *Experimental result*

The results obtained are shown in Table 3.

Table 3. Change in the size of the crystals which were left at various temperatures after the crystallization at various cooling velocities

Cooling / Leaving days	Left at room temp. after rapid cooling	Left at room temp. after slow cooling	Left at 37°C after rapid cooling	Left at 37°C after slow cooling
0	32×8 ~ 130×16	36×8 ~ 250×80	36×8 ~ 110×20	49×16 ~ 130×16
3	49×25 ~ 320×98	49×32 ~2200×410	25×16 ~ 800×250	65×32 ~ 750×410
10	32×16 ~ 370×98	58×80 ~1500×580	32×16 ~1000×320	32×16 ~1500×510
20	32×16 ~ 490×250	32×25 ~2600×450	32×16 ~ 950×290	49×49 ~ 880×250
30	49×32 ~ 490×260	49×32 ~2600×560	32×16 ~ 960×320	98×65 ~ 980×490
60	49×32 ~ 720×320	49×49 ~2500×800	32×16 ~1300×490	112×65 ~1280×560
90	32×32 ~ 980×450	98×82 ~2600×500	49×49 ~1300×490	98×65 ~1400×560

As seen in Table 3, the size of the crystals which were formed by rapid cooling are smaller than that by slow cooling.

When the formed crystals were kept at room temperature or at 37°C, the size of the crystals which were small because of rapid cooling did not become larger than that those resulting from slow cooling, even if the small crystals were kept at higher temperature (37°C). That is to say, if the size of the crystals which are of comparatively larger size at the initial generation continue so, in comparison with those which were small at the initial generation. After all, the initial size of the formed crystals is the most important factor.

Summary

The changes in the shape and size of the crystals during storage depend on the rate of mixing of the solutions containing the chemical components, the concentration of the

solutions and the storing temperature. When the mixing of the solutions is done rapidly, the changes in the size and form of crystals are great and when the mixing is done slowly (in two minutes), the changes are slight and little.

The higher the concentration of the mixed solution is, the smaller the size and the larger the number of the floating crystals. From the more dilute solution, a less number of larger crystals attaching to the inside wall of the container generate. The same results were obtained from the heated mixed solution.

When the mixture solution of the component ions is heated and then cooled, the size of the crystals formed is different from those formed at different cooling velocities. If the mixture is rapidly cooled, the size of the crystals is small and the number is great. And if the mixture is slowly cooled, the size is larger and the number is a few. When those crystals which have been small are stored, their size does not become larger than the crystals which have been formed originally in larger size.

That is to say, the increase of the size of the crystals is principally influenced by the initial size of the crystals formed.

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いか食中毒の生化学的研究

第1報　貯蔵中に於けるアミン類の変化

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Biochemical Studies on Squid Meat Food Poisoning

I. Identification of amines in squid meat during the period of preservation.

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Abstract

In this paper, the authors intend to report investigation on the biochemical changes connected with food poisoning, and to obtain knowledge on the amines which play an important part in food poisoning.

Three kinds of samples were prepared —— all were kept at 11.5°C, 18.5°C and 27.0°C. During preservation the changes on the surface of meat, pH value and volatile basic nitrogen of trichloroacetic acid extractives were observed. The produced amines of the samples were also determined every day by chromatography. The following amines are detected; putrescine, cadaverine, ammonia, monomethylamine, trimethylamine, isoamylamine, β-phenethylamine, histamine, tyramine, octopine, agmatine, arcaine, creatinine and methylguanidine.

従来食物中毒の原因究明に当つては，発症後当時と状態を異にする原因食に就いて検索が行われているが，其の性質上之等に対して充分なる基礎研究が行われているものでなければ，早急に原因把握が出来ないものである。

特に食物中毒の大部分が水産食品であり，軟体動物に依る中毒例が著しいこと，更にイカの如き全國的に漁獲の大にして，又蛋白資源として重要な役割をなしているものに就いては，一層其の感を深めるものである。依つて玆に中毒に関係の多いアミンに就いて試験を行い，其の一端を明らかにして，今後の研究の一助にせんとして此の試験を行つたのである。

実　験　の　部

1. 試料

函館沖にて漁獲したスルメイカ *Ommastrephes sloani pacificus* (STEENSTRUP) を用いた。其の一般組成は水分 77.85%，粗蛋白 17.94%，灰分 2.93%，脂肪 1.24%で此の18尾を次の3群に分け，夫々下記の処理を施した。

第1群：試料6尾を採り，外套膜切開，内臓除去後水洗し，表面に付着せる水分を濾紙で拭き取つて屋外に吊し乾燥した。平均気温は11.5°C (8.5°〜12.5°C)，相対湿度の変域は50〜58%であつた。

第2群：試料6尾を上記同様処理した後バット上に展延し，屋内に乾燥した。平均温度は18.5°C (15.5°〜21.5°C) で相対湿度は55〜70%の変域を示した。

第3群：試料6尾を上記同様処理し，バット上に展延し，27°Cの恒温器内に放置乾燥した。相対湿度の変域は43～59%であつた。

2．実験方法

上記各群より毎日一定時刻に1尾宛同時に採り出し，感能試験後頭脚部を除去し，刻細均質とし，pH及び揮発性塩基窒素量を測定し，尚アミン類の定性試験を行つた。

1）感能試験

各試料の貯蔵中の状態を連日試料採取の際に色，匂，肉表面の状態，食品価値等に就いて観察した。

2）pHの測定

厚生省衛生検査指針[1]に依り試料を調製し，Beckman Model H. 2 Glass Electric pH Meter を用いて測定を行つた。

3）揮発性塩基窒素量の測定

上記厚生省衛生検査指針[2]に依り行い，蛋白沈澱剤には三塩化醋酸を供試した。

4）アミン類の定性試験

佐竹[3]氏の方法に準拠して行つた。

i）揮発性アミン

刻細試料3g宛を採取し，100ccの水と共に15分間ホモゲナイザー処理，30ccの水を添加攪拌後10%NaOH 5ccを加え直ちに水蒸気蒸溜を行う。溜分はIN HCl 5ccに捕集し，約100ccに達すれば蒸溜を止めて之を湯浴上に蒸発乾涸する。残渣を水0.5ccに溶解して0.05cc宛6枚のペーパークロマト濾紙に塗沫し，一次元上昇法に依り20°±2°Cでペーパークロマトグラフイーを行つた。展開溶媒はブタノール(5)：醋酸(2)：水(2)（容積比）を用い，展開乾燥後1枚をネスラー試薬，1枚を芥子油反応で夫々アンモニア，一級二級アミンを検出し，残りの4枚をニンヒドリン法で呈色せしめ，各スポットのRfを決定後各アミンの純品と試料を混和展開して同定確認を行つた。

ii）不揮発性アミン：イミダゾール・オルトフエノール化合物

刻細試料5g宛を秤取し，之を10ccの水と共にホモゲナイザー処理を行い，IN HClでpH2.5とし，95%アルコール50ccを加えて良く攪拌した後30分間遠心する。上澄を湯浴上に濃縮して得た残部を水4ccに溶解し，其の0.05cc宛をペーパークロマト法で展開した。展開溶媒はINアンモニア水を飽和したブタノールを用い，展開後風乾し15分間50°～60°Cに加温してアンモニアを除いてからヂアゾ法で発色した。各スポットのRfは各試料共5枚のペーパーストリップの平均値で表わし，各アミンの純品と試料を混和展開して同定確認を行つた。

iii）不揮発性アミン：グアニヂン化合物

試料はイミダゾール化合物検索に用いたものを供試し，前回同様の方法でペーパークロマトグラフイーを行つた。展開溶媒はブタノール(4)：醋酸(1)：ピリヂン(1)：水(2)容量比で，発色は牧[4]氏の坂口反応に依つて行つた。Rfはペーパーストリップ4枚宛の平均値として表わし，前回同様に同定確認を行つた。

実験結果

第1表はスルメイカ肉放乾中の状態変化を，第2表はpHの変化を第3表は揮発性塩基窒素量の変化を示したものであり，尚第4表はペーパークロマトグラフイーに依り確認したアミン類の発現を表示し，第2図，第3図及び第4図，2，3は之等を図示したものである。

即ち第1表から明らかな如く，スルメイカを外套膜切開，内臓除去して27°Cに展延放乾した試料は，平均気温11.5°Cの屋外に乾燥した試料が実験期間中終始可食の状態を持続したのに比べ，48時間以後は口にすること不可能となり，乾燥終了後も外観悪く悪臭を放ち，スルメとして最下級品であつた。

又放乾中のpHの変化は第2表，第1図に示す如く，27°Cに放乾したものは他に比べて常にアルカリ側に

Table 1. Changes of appearance of spuid meat

Sample No. (Mean temp. of preservation) \ Time elasped (in hrs.)	0	24	48	72	96	120
Group 1 (11.5°C)	eatable	eatable	eatable	eatable	eatable	eatable
	light red	white	light red	light red	light reddish gray	light reddish gray
	semitransparent	semitransparent	opapue	semitransparent	semitransparent	semitransparent
			fragrant	fragrant	special fragrant as Surume	special fragrant as Surume
	soft and elastic meat	soft and elastic meat	elastic meat	tight and elastic meat	tight meat	tight meat
Group 2 (18.5°C)	same as group 1	eatable	eatable by heating	eatable by heating	eatable by heating	eatable by heating
		light red	light turbid red	white	turbid white	turbid light gray
		opaque	opaque	opaque	opaque	opaque
		light fishy	fishy	fishy	very fishy	very fishy
		soft and elastic meat	soft and elastic meat	soft and elastic meat	soft meat	very soft meat
Group 3 (27.0°C)	same as group 1	eatable by heating	eatable by heating	eatable by heating	no eatable even by heating	no eatable even by heating
		turbid white	turbid white	light yellowish white	reddish gray	reddish gray
				opaque	opaque	opaque
		ill-smell like ammonia	ill-smell like ammonia	very ill-smell like amines	ill-smell like amines	ill-smell like amines
		very soft meat	soft meat	soft and adhesive meat	hard, thin and flat meat	hard, thin and flat meat

Table 2. Changes of pH of squid meat

Sample No. (Mean temp. of preservation) \ Time elasped (in hrs.)	0	24	48	72	96	120
Group 1 (11.5°C)	5.90	6.31	6.01	6.09	6.01	6.30
Group 2 (18.5°C)	5.90	6.50	6.73	6.41	6.88	7.57
Group 3 (27.0°C)	5.90	6.80	7.53	7.38	7.27	7.58

Table 3. Changes in the amount of volatile basic nitrogens of squid meat

Sample No. (Mean temp. of preservation) \ Time elasped (in hrs.)	0	24	48	72	96	120
Group 1 (11.5°C)	8.91	7.44	5.06	10.13	15.74	55.97
Group 2 (18.5°C)	8.91	12.14	18.88	27.07	61.45	121.70
Group 3 (27.0°C)	8.91	174.95	227.49	242.70	303.97	356.57

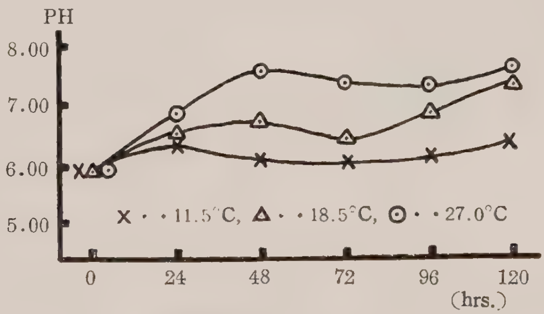


Fig. 1. Changes of pH values of squid meat

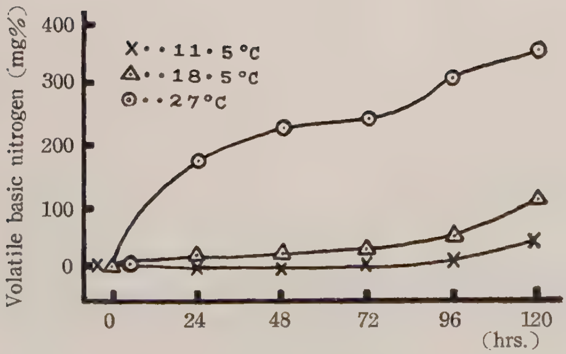


Fig 2. Changes in the amount of volatile basic nitrogens (mg%)

Table 4. Increase and decline of amines of squid meat

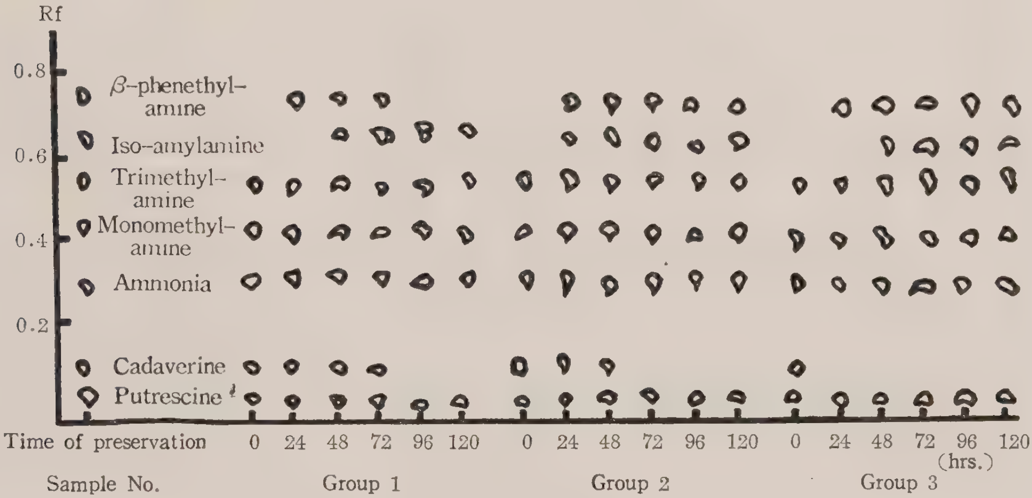
Fraction of amines	Sample No. (Mean temp. of preservation)	Group 1 (11.5°C)						Group 2 (18.5°C)						Group 3 (27.0°C)					
	Time elasped (in hrs.)	0	24	48	72	96	120	0	24	48	72	96	120	0	24	48	72	96	120
Volatile amines	putrescine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	cadaverine	+	+	+	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−
	ammonia	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	monomethyl-amine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	trimethyl-amine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	isoamyl-amine	−	−	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+
	β-phenethyl-amine	−	+	+	+	−	−	−	−	+	+	+	+	−	+	+	+	+	+
	number of unknown substances	0	2	2	3	1	1	0	3	5	5	4	3	0	1	1	1	1	1
Imidazol and ortho-phenol compds.	histamine	−	−	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+
	tyramine	−	−	+	+	+	+	−	−	+	+	+	+	−	−	+	+	+	+
	number of unknown substances	1	1	2	2	2	1	0	1	4	4	4	5	1	1	2	2	3	2
Guanidine compds.	octopine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	agmatine	−	−	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+
	arcaine	−	−	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+	+
	creatinine	−	−	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+	+
	methyl-guanine	−	−	−	−	+	+	−	+	+	+	+	+	−	+	+	+	+	+
	number of unknown substances	0	0	0	2	0	0	0	0	1	2	3	3	0	2	2	0	1	1

傾いて居り，最初pH5.9であつたのが48時間後にpH7.5と極大値を示し，後降下するが120時間後に再び上昇してpH7.6を示している。11.5°C放乾のものは24時間後にpH6.3となるが其の後降下し，96時間後から再び僅かに上昇するに至るも，おゝむねpH6.0を中心としている。尚18.5°C放乾試料は常に両者の中間に位して変化し，48時間後にはpH6.7を示すが72時間後にはpH6.4と成り，其の後は再び上昇して120時間後にはpH7.6を示す。然しながらいずれの試料も其のpHは乾燥初期24〜48時間に極大値を示した後一度低下し，再び上昇の傾向を示している。

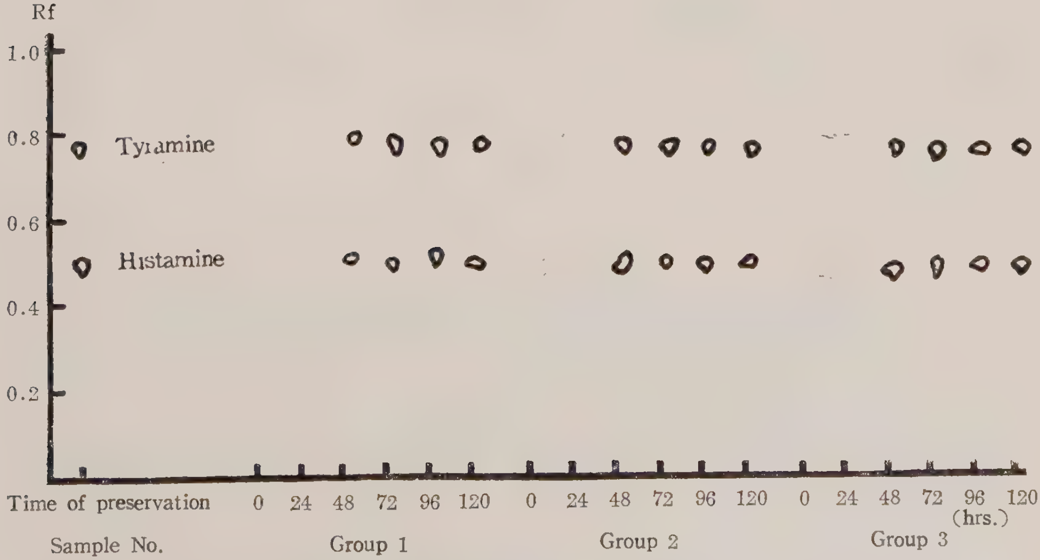
揮発性塩基窒素量の変化は第3表，第2図に示す如く，27°C放乾試料は他に比べて非常に多く，乾燥初期

に急激に増加し，其の後一時増加率は停滞するが再び増加して120時間後は360mg%に達する。11.5°C放乾試料は48時間以後増加を来たすが，120時間後に於いても約60mg%に達するに過ぎなく，18.5°C放乾試料は乾燥の進行と共に漸増し120時間後には約120mg%で前者の約2倍量に達する。

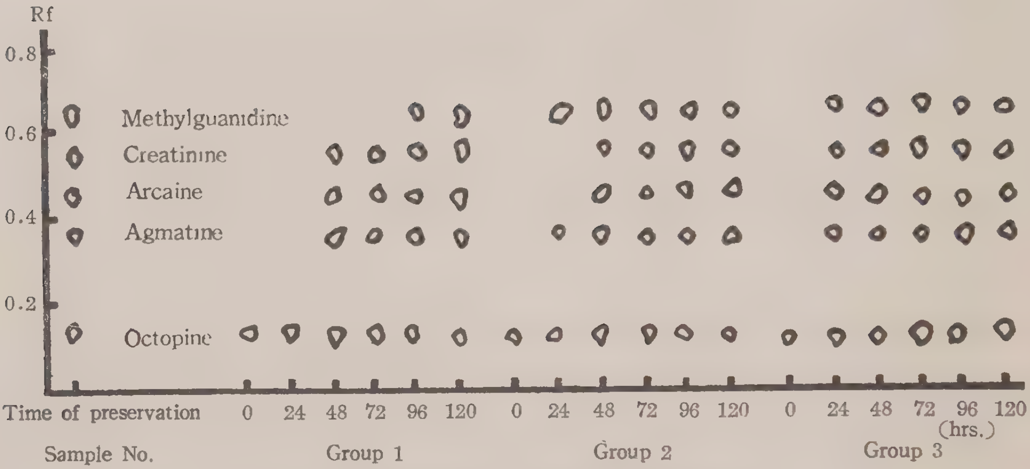
而して此の間に於けるアミン類の消長は第4表，第3図1，2，3に示す如く，全試料を通じて確認されたものとしてプトレスチン，カダベリン，アンモニア，モノメチルアミン，トリメチルアミン，イソアミルアミン，β−フエネチルアミン，ヒスタミン，チラミン，オクトピン，アグマチン，アルカイン，クレアチニン及びメチルグアニヂンがあり，猶此の外に未確認のスポットがあつた。而して此の中ヒスタミンの生成が



(1) Amines detected by Nessler's indicator, dithiccarbamate reaction and ninhydrin reaction



(2) Amines detected by diazo reaction



(3) Amines detected by Sakaguchi reaction

Fig. 3. Paper chromatography of amines of squid meat

徼アルカリ乃至は弱アルカリ性下で認められたが，此の点に就いては先に木俣，河合[5]両氏のヒスタミンは必ず酸性側で行われると述べている点と幾分相違するも，而しイカ肉蛋白の如き各種酵素の混在する処ではヒスタミン生成の至適水素イオン濃度は上昇するものと考えられる。又此のヒスタミン生成がイカ肉中の酵素作用に依るものか，付着細菌の作用に依るものかは明らかでないが今後此の点に就いても究明の必要があると思われる。

要　　約

内臓を除去したスルメイカ筋肉を11.5°C，18.5°C及び27.0°Cの各温度に放乾し，其の状態変化を感能試験に依り，又胴肉のpH及び揮発性塩基窒素量の変化を定量的に検索し，同時に生成されるアミン類をペーパークロマトグラフイーに依り検索確認した。

検索し得たアミン類は全試料を通じてプトレスチン，カダベリン，アンモニア，モノメチルアミン，トリメチルアミン，イソアミルアミン，β-フエネチルアミン，ヒスタミン，チラミン，オクトピン，アグマチン，アルカイン，クレアチニン及びメチルグアニヂンであつた。

本研究は文部省科学研究費に依り遂行した。謝意を表する。

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いか食中毒の生化学的研究

第2報　生成アミン類の消長に就いて

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Biochemical Studies on Squid Meat Food Poisoning

II. Increase and decline of amines of squid meat during preservation.

Kiichi MURATA, Keiichi ÔISHI and Atsushi IIDA

Abstract

The authors have previously identified many amines accumulated during the period of preservation of squid meat. In this paper, are reported quantitative analyses of three amines: trimethylamine, histamine and tyramine, comparing samples with the pure reagents chromatographically.

General speaking, the inclination was observed for trimethylamine to increase in accordance with the increase of the volatile basic nitrogen, and the higer the temperature at which the samples were preserved, the larger became the quantity of trimethylamine determined. A slight amount of histamine was detectable after 96 hours from the catching time for all the samples, therefore it seems not to play any important part in the food poisoning caused by the squid meat. Tyramine content was slight and increased gradually.

著者等は各種条件下にスルメイカを乾燥した場合の，其の筋肉中のアミン類の消長をペーパークロマトグラフイーに依つて定性的に認めたが，之等の中で最も中毒機構に関聯を有し且つ定量可能なトリメチールアミン，ヒスタミン及びチラミンに就いて各々其の生成変化を観察した。

実験の部

1. 試料

供試スルメイカ *Ommastrephes sloani pacificus*（STEENSTRUP）は函館沖にて，漁獲せるもので，水分77.85%，粗蛋白17.94%，灰分2.93%，粗脂肪1.24%の一般組成を有する18尾を3群に分け，夫々下記の処理を施した。

第1群：6尾を採り外套膜切開，内臓除去後水洗し，表面に付着せる水分を濾紙で拭き取つて屋外に吊し乾燥した。平均気温は11.5°C（8.5°～12.5°C）で相対湿度の変域は55～58%であつた。

第2群：上記同様処理したもの6尾をバット上に展延し室内に放置乾燥した。平均気温は18.5°C（15.5°～21.5°C）で相対湿度の変域は55～70%であつた。

第3群：上記同様処理したもの6尾をバット上に展延し，27°Cの恒温器内に放置乾燥した。相対湿度の変域は43～59%であつた。

2. 実験方法

上記各群より毎日一定時に1尾宛採取，刻細し，其の一部を採つて先に推知し得たアミン類の中で定量法の確立しているトリメチールアミン，ヒスタミン並びにヒスタミン同様ヂアゾ反応を呈するチラミンに就い

て比色定量を行つた。

1) 定量用試料の調製

厚生省衛生検査指針[1]に依り各試料の抽出液を調製し，之を佐竹[2]氏のクロマト吸着法で処理して得た塩基部を水蒸気蒸溜法及び溶媒分別法で，トリメチールアミン区，ヒスタミン区及びチラミン区に分別した。此の分離並びに分別法に依る回収率はトリメチールアミン93±2％，ヒスタミン86±3％，チラミン88±3％である。操作は次の如く行つた。

Amberlite IRC-50の100～120meshのものを径1cmのビューレットに高さ15cmに充塡し，50ccの2N NaOHで毎分1.5ccの流速で洗滌する。次にIN醋酸緩衝液（pH 5.0）80ccを毎分1.5ccの流速で通し，最後に水100ccで洗滌してカラムをpH5.0とする。

此のカラムに先に調製した抽出液100ccを毎分1.5ccの流速で通し，水50ccで洗滌，流下液，洗液を棄てる。次にIN HCl 30ccを毎分1.5ccの流速で通じた後水20ccで洗滌する。此の流下液，洗液を合して採る。カラム洗滌時の流速は毎分3.5ccとした。得られた流下液，洗液の混液はIN NaOHでpH 9～10として水蒸気蒸溜を行い，溜分を20ccのIN HClで捕集し溜液，捕集液合して約300ccに成れば蒸溜を止める。之を湯浴上に濃縮し，水を加えて一定量とし，トリメチールアミンの定量に供した。水溜後の残液は完全に回収して湯浴上に蒸発乾涸，水に溶解して20ccとし50cc容分液濾斗に移し，n-アミールアルコール10cc宛で3回，1回につき200回宛振盪して抽出してアミールアルコール層と水層に分つ。アミールアルコール層はヒスタミン含有区で，水層はチラミン含有区である。アミールアルコール層は合して10％HCl 10cc宛で3回抽出を行い水層を合し濃縮後又はそのまゝ水を加えて一定量とし，ヒスタミンの定量に供した。チラミン含有の水層はIN HClで中和し，10ccアミールアルコールを添加，Na_2CO_3アルカリ性下（pH9～10）前回同様3回宛抽出を行う。アルコール層を合して10％ HCl 10cc宛3回抽出後，水層を合してチラミン含有区とする。之をヒスタミン区の場合同様一定量とし供試した。

2) 定量方法

定量方法は次の如くであるが，定量値は試料乾燥中の水分減量を算出し，元重量に対する値として計算した。

i) トリメチールアミンの定量

Dyer[3),4)]のトリメチールアミン・ピクラート法に準じて行つたが，吾々の得たトリメチールアミン区は過剰の遊離塩酸を含むから，トルオール抽出に際し，過剰の固型Na_2CO_3を用いた。比色は Beckman 型 Spectrophotometer に依り波長410mμのfilterを用いて行い，定量値はトリメチールアミンmg％として示した。

ii) ヒスタミンの定量

供試々薬の調製及び定量時の呈色法は木俣・河合[5)]両氏のヂアゾ法に依り行つた。両氏は比色に際しデュボスク比色計を用いているが，著者等は AKA 型 Electrophotometer を用いた新たな方法で，其の filter No. 3（波長 470mμ）を使用してヒスタミン二塩酸塩の標準曲線を作製し，之を用いて定量値を算出した。此の標準曲線の定量範囲は0～80γである。定量値はヒスタミン mg％ で示した。

iii) チラミンの定量

ヒスタミン同様ヂアゾ反応で呈色せしめ，AKA 型 Electrophotometer の filter No. 3を用いて定量を行つた。得られた標準曲線のチラミン塩酸塩の定量範囲は0～100γである。定量値はチラミン mg％で示した。

実験結果及び考察

第1図はトリメチールアミン・ピクラートの標準曲線を示したものであり，第1表，第2図は検体中のトリメチールアミン含量を，又第3図はヒスタミン二塩酸塩の標準曲線，第2表，第4図は同じく検体中のヒスタミン含量を示し，第5図はチラミン塩酸塩の標準曲線で，第3表第6図は同様検体中のチラミン含量の変化を求めたものである。

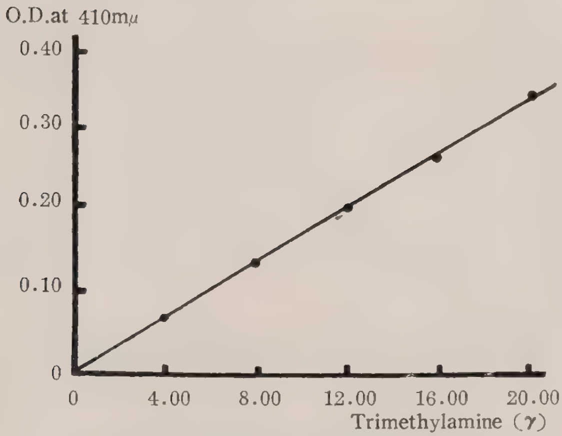


Fig. 1. Relation between the concentration of trimethylamine-N and optical density

Table 1. Changes in the amount of trimethylamine of squid meat (mg%)

Sample No. (Mean temp. of preservation) \ Time elasped (in hrs.)	0	24	48	72	96	120
Group 1 (11.5°C)	4.09	4.50	4.71	3.60	3.34	6.76
Group 2 (18.5°C)	4.09	5.94	14.27	21.11	24.09	27.59
Group 3 (27.0°C)	4.09	5.41	32.68	42.41	56.41	63.70

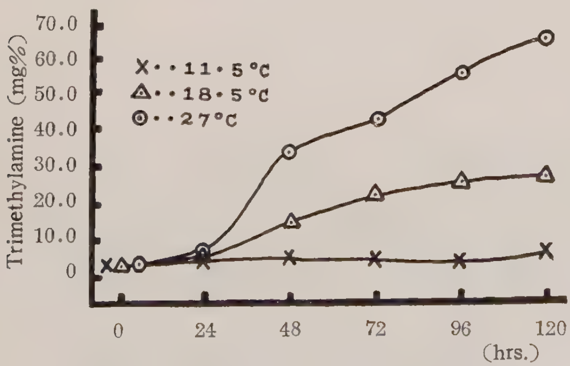


Fig. 2. Changes in the amount of trimethylamine of squid meat

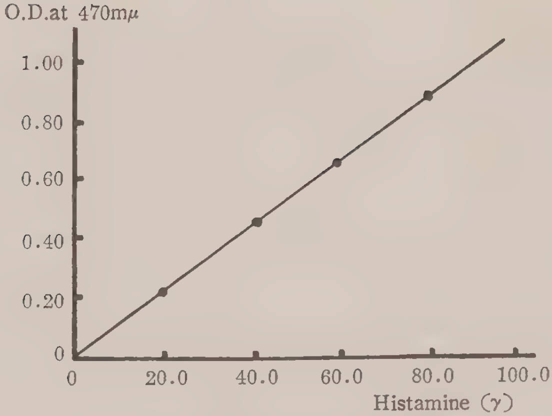


Fig. 3. Relation between the concentration of histamine-dihydrochloride and optical density

Table 2. Changes in the amount of histamine of squid meat (mg%)

Time elapsed (in hrs.) / Sample No. (Mean temp. of preservation)	0	24	48	72	96	120	144	168
Group 1 (11.5°C)	0	0	0.25	0.31	0.29	0.33	0.48	0.47
Group 2 (18.5°C)	0	0	0.31	0.46	0.40	0.50	0.65	0.57
Group 3 (27.0°C)	0	0	0.59	1.04	3.67	1.85	1.59	0.76

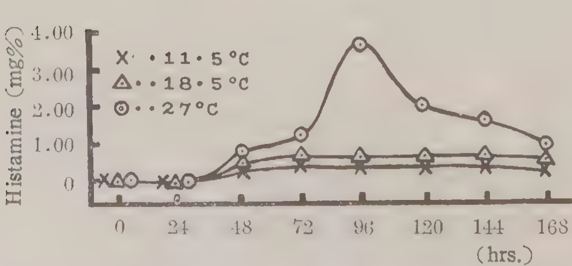


Fig. 4. Changes in the amount of histamine of squid meat

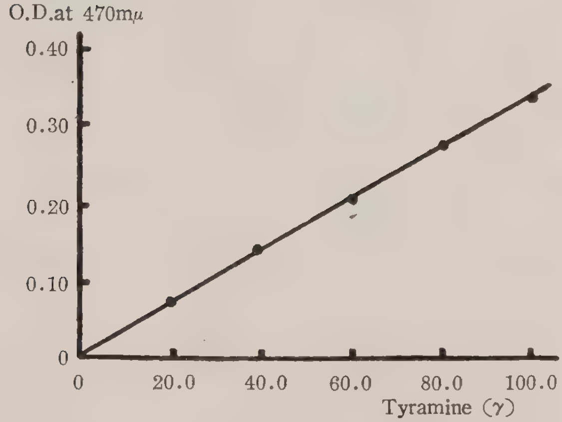


Fig. 5. Relation between the concentration of tyramine-hydrochloride and optical density

Table 3. Changes in the amount of tyramine of squid meat (mg%)

Sample No. (Mean temp. of preservation) \ Time elasped (in hrs.)	0	24	48	72	96	120
Group 1 (11.5°C)	0	0	1.69	1.09	1.05	1.08
Group 2 (18.5°C)	0	0	2.24	1.44	1.19	1.61
Group 3 (27.0°C)	0	0	3.46	1.72	2.39	2.69

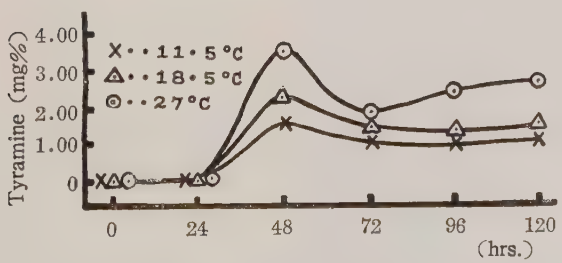


Fig. 6. Changes in the amount of tyramine of squid meat

即ち第1表より，トリメチールアミン生成量は24時間以内は温度の高低其の他の条件に依つて殆んど変化をみないが，48時間以後にあつては温度高きものは次第に上昇し，120時間後に，18°C 放乾試料にあつては 27.6mg%，27°C放乾のものは 63.7mg%に達する。然るに 11°C放乾試料は 120時間の経過に依つても僅かに 6.8mg%に達するに過ぎなく，殆んど其の生成に変化が見られない。

ヒスタミンにあつては，何れも其の生成は極めて少なく，11°C 及び 18°C放置試料にては 168時間の経過に依つても僅かに 0.5〜0.6mg%以下に過ぎなく，僅かに 27°C 放乾のものが 96時間前後に 3.67mg% に達するも其の後減少して 168時間に於いては 0.76mg%と成る。

又チラミンにあつては次第に増加の傾向を示すが，各々の生成量も少なく，高温の 27°C放乾のものにあつても 120時間目で 2〜3 mg%に達するに過ぎない。

要　　約

① 内臓除去したスルメイカ筋肉を 11.5°C，18.5°C 及び 27°C の各温度に放乾し，トリメチールアミン，ヒスタミン及びチラミンの消長を定量的に求めた。

② トリメチールアミンは高温に放乾せられるものが急激に生成量を増加する。

③ ヒスタミンは高温のもの 27°C 放乾試料に於いては 100時間前後の経過に依り 3 mg%に至るも，其の後減少し，凡て生成量は極めて少い。

④ チラミンは何れも漸増の傾向を示し，温度の変化に依り生成量に著しい差異は認められない。

本研究は文部省科学研究費に依り遂行した。謝意を表する。

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LIMNOLOGICAL RECONNAISSANCE OF LAKE NUKABIRA, A RESERVOIR IN HOKKAIDO

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Lake Nukabira, located in Nukabira, Kamishihoro, Eastern Hokkaido, is a body of water impounded by a dam constructed in connection with a hydro-electric generation plant. This reservoir was filled up with water in September, 1955; in June of the next year Eguchi and Fujioka (1956) made a preliminary observation on the general limnology of this lake. The present investigations attempting also a general survey of the limnology of the present reservoir were carried out on November 15–17, 1956.

The authors wish to express deep gratitude to Prof. S. Motoda of the Faculty of Fisheries, Hokkaido University, for his generosity in giving many invaluable suggestions during the present work. They also express their grateful thanks to Prof. H. Takano and Prof. K. Shimakura of the Obihiro Zootechnical University for their favours in providing facilities for the present research.

Outline of Physiographic Features of the Lake

The dam as constructed is 76 meters in height and 287 meters in length; the reservoir occupies an area of 8.1 km^2; the volume of impounded water is 19.4 km^3 (Fig. 1). The long axis of the lake extends about 8 km from north–west to south–east, the greatest width measuring about 3 km in the southern part of the lake. The shoreline shows a high degree of irregularity. The deep basin is generally found along the long axis from north–west to south–east above the old bed of the River Otofuke. The maximum depth of 75 m occurs in the southern part of the lake near the dam. In the present observation, though no exact topographic measuring was done, the depth of the lake was recorded as 12 m at St. 1, 19 m at St. 2, 38 m at St. 3 and St. 4, 48 m at St. 5, 50 m at St. 6 and 73 m at St. 7 respectively (Fig. 1).

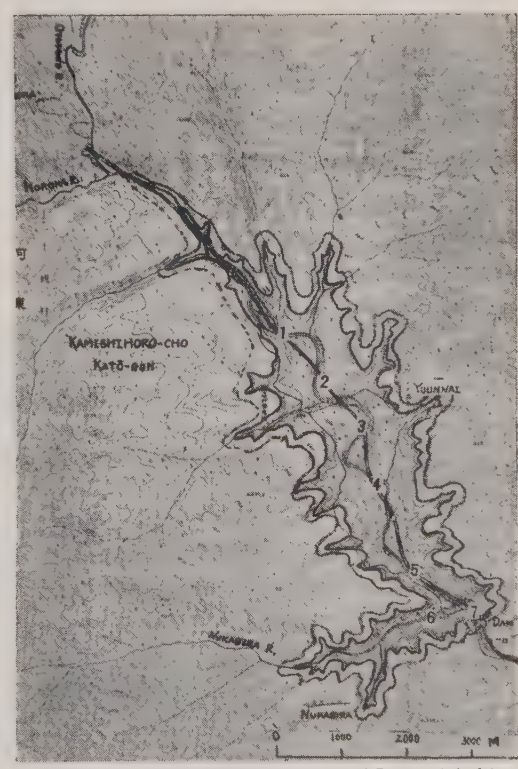


Fig. 1. Map showing location of Lake Nukabira and the stations at which observations were made

There are several rivers flowing into the lake basin; and they drain a total area of 378 km². Of these, the Otofuke River on the northern-most shore is of considerable importance as one of the sources of the lake water.

Physical and Chemical Properties of the Lake Water

Field observations were made at seven stations, but observations on physical and chemical properties of the lake water were made only at St. 2 and 6.

Station	Date	Hour	Air temp. (°C)	Weather
1	Nov. 15, '56	2:50–3:00 p. m.	−3	Snow showers
2	〃	3:30–5:30 p. m.	−3	〃
3	Nov. 16, '56	10:30–10:50 a. m.	−4	Overcast with lower clouds
4	Nov. 17, '56	10:20 a. m. – 12:00 m.	−2	Snow
5	Nov. 16, '56	11:20–11:30 a. m.	−4	Snow showers
6	〃	11:50 a. m. – 12:20 p. m.	−4	〃
7	〃	1:25–3:00 p. m.	−3	Overcast with lower clouds

Temperature of water: The temperature of water from the surface to near the bottom ranged vertically from 6.67 °C to 7.47 °C at St. 2 and from 7.05 °C to 7.88 °C at St. 6 as shown in Table 1 and Fig. 2. The difference of temperature between the

Table 1. Physical and chemical properties of the water observed on Nov. 15–16, 1956

Depth (m)	Temp. of water (°C)		Dissolved oxygen (cc/1)		Dissolved oxygen (%)		pH		Chlorine (mg/1)		Half-bound carbon dioxide (mg/1)		KMnO₄ consumption (mg/1)		Silicate-Si (μg-atoms/1)		Phosphate-P (μg-atoms/1)	
	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6	St. 2	St. 6
0	6.67	7.57	6.11	6.52	76.8	79.6	6.9	6.8	6.5	6.5	18	19	1.4	1.3	30.3	50.0	0	0
2	7.49	7.70	6.48	5.83	83.1	75.0	7.2	6.8	—	6.0	—	—	—	1.4	35.7	50.0	0	0
4	7.71	7.83	6.03	6.00	77.6	77.4	6.9	6.8	6.0	6.5	—	—	1.5	1.4	31.6	44.4	0	0
6	7.28	7.64	6.01	5.90	76.7	75.7	6.9	6.8	—	—	—	—	—	—	40.0	48.9	0	0
8	7.20	7.88	6.25	5.71	79.5	73.9	6.9	6.8	6.5	5.6	—	—	1.6	1.3	44.0	47.0	0	0
10	7.40	7.69	7.54	5.75	95.8	74.0	6.9	6.8	—	—	—	—	—	—	44.4	47.0	0	0
12	7.42	7.88	6.98	5.90	89.3	76.3	6.85	6.8	7.0	6.0	—	—	1.6	1.3	40.0	54.8	0	0
14	7.49	7.70	7.99	5.61	102.4	72.2	6.8	6.8	—	—	—	—	—	—	40.0	51.3	0	0
16	7.47	7.88	6.40	5.97	82.1	77.2	6.8	6.8	7.0	5.6	17	17	1.6	1.3	40.0	57.1	0	0
18		7.70		5.82		74.9		6.8		—		—		—		57.1		0
20		7.78		5.66		73.0		6.8		6.0		—		1.3		51.3		0
25		7.86		5.74		74.3		6.9		—		—		—		51.3		0
30		7.81		5.55		71.6		6.9		5.6		—		1.3		32.0		0
35		7.70		5.74		73.9		6.9		—		—		—		28.0		0
40		7.05		5.11		64.8		6.7		6.0		—		1.3		30.0		0
45		7.62		4.11		52.8		6.7		6.0		17		1.4		26.0		0
50																		

Transparency (m)	St. 2	St. 6	Color of water (No.)	St. 2	St. 6
	3.0	3.0		10	10

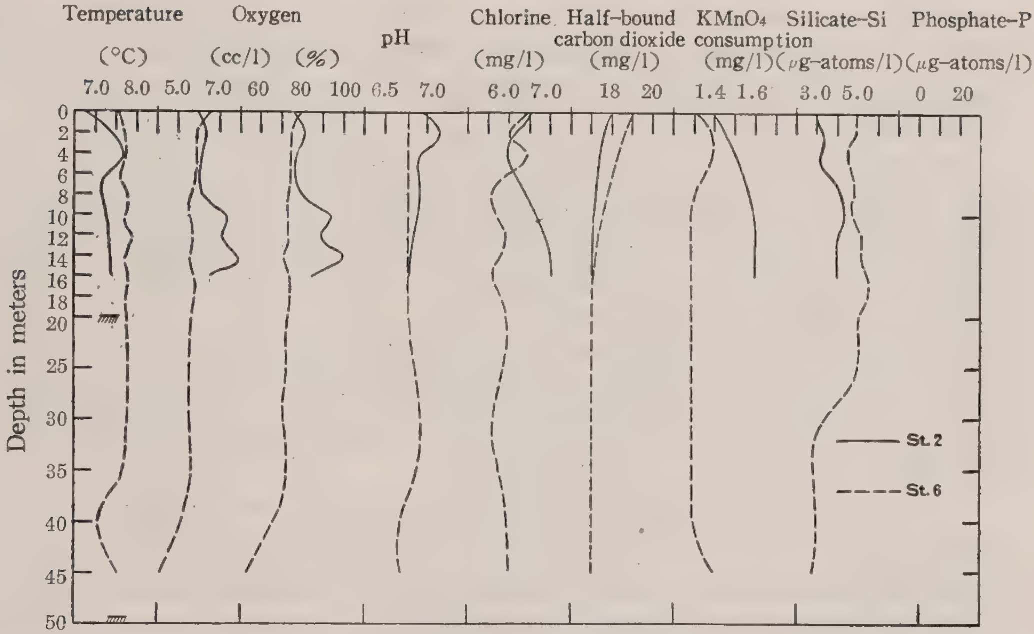


Fig. 2. Hydographic conditions at St. 2 and St. 6

surface and the bottom water was very small, no thermocline being formed. In detail, however, a slightly poikilothermic condition was formed possibly on acount of partial circulation which had occurred with the decrease of surface temperature.

As is expected from the figure of the vertical distribution of water temperature, at the present time the bottom water in the deep region (St. 6) would not be subjected to any influence by the water of upper layers.

Horizontal differences of the water temperature, though very small, were found between the two stations, 2 and 6; the temperature of water showed lower values at St. 2 through all layers. Such a low temperature at that station was possibly caused by the inflow of water of low temperature from the Otofuke River.

Color and transparency of water: The color of lake surface as read by Forel's scale was very high, showing No. 10 at both stations. The lake water presented the yellow brown color probably due to the tint of the suspended materials, but the lake water filtered by filter paper was very transparent. The present lake will be disignated from its color as a lake of common type such as is widely formed in Japan.

The transparency of water was comparatively low, being 3 meters of Secchi's disc reading at the two stations. The low transparency of this lake seems to be brought about by the large crop of plankton, mainly composed of *Asterionella formosa*.

Dissolved oxygen: The content of dissolved oxygen ranged vertically from 7.99 cc to 6.01 cc at St. 2 and from 6.52 cc to 4.11 cc at St. 6. The oversaturated oxygen was

found only at 14 meters layer at St. 2; as a whole, the oxygen was dissolved in the range of 70-80% saturation. This fact is very different from the condition of such a bog lake as the Uryu Reservoir, in which there is only 30% saturation of oxygen at 2 meters depth (Motoda, 1950).

In some mountain lakes of northern Japan a complete mixing of lake water often does not occur even in the autumnal circulation period according to their morphological patterns. The gradient of vertical distribution of oxygen in the present lake offers proof of fact that deeper layer was not influenced yet by a complete circulation of the upper water in the period of autumnal overturn.

pH value: The pH value showed a range of 7.2 to 6.8 at St. 2 and of 6.9 to 6.7 at St. 6 respectively. The vertical distribution of the pH value was almost uniform from the surface to the bottom, with exception at St. 6. The pH value at St. 2 was somewhat higher than that of St. 6 in the upper layers.

There exists a brimstone-mine at the upper course of the Horoka River, one of the branch streams of the Otofuke River. Therefore, it is possible that SO'_4 coming out from the soil makes the river water acidic by the action of H_2SO_4, and, in turn that causes the lake water also to be acidic.

Chlorine: It was expected that the water of this lake would contain a comparatively large amount of chlorine because there are the Nukabira and the Yuunnai Hot Springs near the shore of the lake, but the measurement showed such comparatively low chlorinity as 7.0-6.0 mg/l at St. 2 and 6.5-5.6 mg/l at St. 6. These values are somewhat small as compared with those of other lakes in northern Japan.

Half-bound carbon dioxide: Half-bound carbon dioxide measured from 18 mg/l to 17 mg/l at St. 2 and from 19 mg/l to 17 mg/l at St. 6. The vertical distribution of this substance was almost uniform from the surface to the bottom, but, in detail, a slightly large amount was found in the upper layer. The quantity of the half-bound carbon dioxide in the present observation corresponds to a quarter of that of the underground water of Akiyoshidai, Yamaguchi Pref. which is a famous example for containing a large amount of this substance in Japan. The present lake will rank as moderate type in Japan in respect of the content of this substance.

Potassium permanganate consumption: $KMnO_4$ consumption of lake water ranged from 1.4 mg/l to 1.6 mg/l at St. 2 and from 1.3 mg/l to 1.4 mg/l at St. 6 respectively. Such relatively small values of $KMnO_4$ consumption of the lake water may be essentially a prominent feature. Eguchi and Fujioka (1956) also reported the value as small as 2.0 mg/l in June, 1956.

Yoshimura (1937) has reported the following classification as a scale in determining the trophic types of lakes on the basis of potassium permanganate consumption.

Oligotrophic type ………… under 10 mg/l ……… Mountain lake

Eutrophic type ·············· 10 to 20 mg/1 ········ Low land lake
Destrophic type ·············· over 20 mg/1 ········ Bog lake

Judging from this classification, the present lake is suggested to be not a bog lake, but a mountain lake with respect to the nature of the water.

Silicate and phosphate: The dissolved silicate-Si was 30.3–44.4 μg-atoms/1 at St. 2 and 26.0–57.1 μg-atoms/1 at St. 6 respectively; phosphate-P came to zero at both stations. The vertical distribution of silicate-Si tended to be present in maximum quantity in the middle layer at both stations. The local difference of these nutrient substances was clear; a considerably small value was observed at St. 2 as compared with that of St. 6. Such a local difference is supposed to have resulted in a remarkable growth of phytoplankton at St. 2 (*Asterionella formosa*). Silicate-Si had declined to but about one-eleventh the content obtained in June of this same year (Eguchi & Fujioka, 1956).

Plankton

Method of collection: Plankton net of ordinary type, with anteriorly tapering canvas cone, 16 cm in diameter at anterior opening, 32 cm in large diameter of the proper filtering portion and 1 m in length of the filtering bolting cloth, was used by hauling vertically from near the bottom to the surface in all stations. In addition, a specially designed quantitative sampler corresponds to 20 liters of water. The collection of plankton with the special sampler was made to ascertain vertical distribution of plankton in detail only at St. 4, which is located at the approximate center of the lake.

Plankton species: The plankton collected represented only 8 species in total. The species identified were as follows:

Cyclops vicinus	r r r
Daphnia longispina	r
Bosmina coregoni	c
Asplanchna priodonta	r
Asterionella formosa	c c c
Volvox aureus	r r
Pandorina morum	+
Eudorina elegans	r

Of these, the dominant species was *Bosmina coregoni* in zooplankton and *Asterionella formosa* in phytoplankton. Above all, the latter species occurred in abundance.

According to the previous observation, the total number of plankton species in this lake counted 6 in June, 1956 (Eguchi & Fujioka, 1956). A copepoda of *Acanthodiaptomus* sp., which appeared in small number in the former observation, was not found at all in the present sampling. The high concentration of phytoplankton comprised of three species of diatoms, of which *Synedra ulna* was dominant, was reported in the former observation,

but these species did not occur at all in the present observation.

The plankton in this lake showed a noticeable difference from other reservoirs in Hokkaido such as the Uryu and the Takadomari. In the Uryu Reservoir filled with water in 1943 the number of species of plankton counted 18 in August, 1946 (Motoda, 1950), and in the Takadomari Reservoir impounded in October, 1953 it was represented by 38 species in October, 1955 and 20 species in October, 1956 respectively (Kurohagi, 1956).

Some investigators (Uèno, 1952; Shiraishi *et al.*, 1953; Round, 1956) stated that a reservoir when impounded by dam construction generally contains a relatively small number of species, but a certain species occasionally grows vigorously, showing nearly monotonous composition. However, the plankton species usually becomes larger with the passage of time as in ordinary lakes. Unusual increase of plankton in the reservoir of a certain time after construction may be induced by the certain nutrient or promoting substances derived from the bottom.

Many observations have shown that a bog lake differs from lakes of ordinary type, eutrophic or oligotrophic, regarding both plant and animal plankton populations. The plankton organisms in bog lakes are usually represented by preponderance of dismids, a small number of diatom species, an abundant occurrence of rotifera and a poverty of copepoda species. Hada (1956) proved the abundance of rotifera in several bog lakes in Hokkaido. The plankton community of this lake observed in the present observation does not show a positive feature of a bog lake.

Among the species observed, the six prevalent species, *Cyclops vicinus*, *Daphnia longispina*, *Bosmina coregoni*, *Asterionella formosa*, *Asplanchna priodonta* and *Pandorina morum* are known as cold-water forms or eurythermal forms. It has been stated that in habit *Cyclops*, *Bosmina*, *Asplanchna* and *Pandorina* occur mainly in cold-water condition of lake, and *Daphnia* and *Asterionella* are known to be eurythermal species. *Asterionella* adjusts to a wide range of temperature of water, showing two types of colony according to the temperature of environment, that is, the cross-shaped forms occur mainly in the cold-water condition. In the present observation the colonies of *Asterionella* were dominantly represented by such cross-shaped individuals.

Vertical distribution of plankton: The vertical distribution of three species of plankton is shown in Fig. 3. *Asterionella formosa*, *Bosmina coregoni* and *Asplanchna priodonta* inhabited all depths. Of these, the former two species were uniformly distributed throughout the layers observed, while *Asplanchna* disappeared entirely at 30 meters layer. The maximum populations were present in the shallow layers, in detail, at the surface in *Asterionella* and *Asplanchna* species and 6 meters layer in *Bosmina*. From this depth downwards, these species decreased in number with depth to the middle layer, though there was some irregularity. Below the middle layer, they commenced to increase and then again decreased near the bottom.

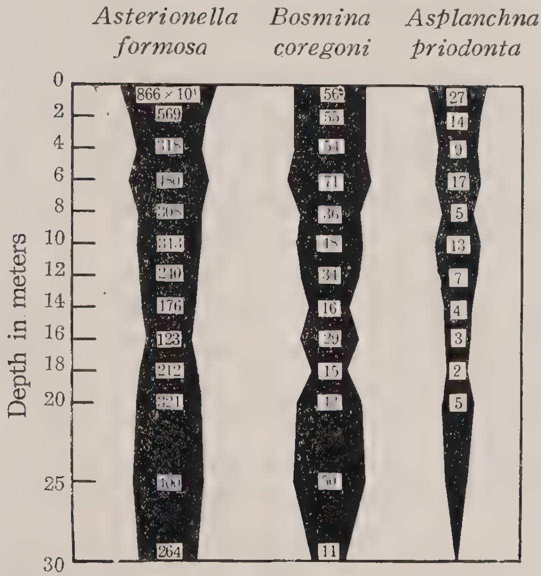


Fig. 3. Vertical distribution of three species of plankton at St. 4

Standing crop of net plankton: The standing crop of net plankton at each station was determined by measuring dry weight and chlorophyll content of the organisms on the samples obtained by the vertical haul with plankton net. The volume of water filtered through the plankton net was calculated by reading of the flowmeter set at the center of the anterior opening of the net. The results are shown in Table 2.

The dry weight of net plankton per cubic meter of lake water ranged from 67 mg to 860 mg. The mean was calculated as 234.5 mg per cubic meter; it corresponds to 561.3 kg per hectare. The organic matter as represented by the weight of ash of plankton organisms was 252.6 kg per hectare (Table 3). The dry weight of plankton somewhat increased to the center region from the north and from the south shore.

Table 3 indicates the dry weight and the weight of organic matter of plankton in several lakes of eutrophic type in Japan.

Table 2. Dry weight and chlorophyll content of plankton observed on Nov. 15–17, 1956

Station	Dry weight of plankton (mg/m³)	Chlorophyll content (mg/m³)
1	77	0.52
2	245	1.36
3	184	—
4	860	2.20
5	95	0.14
6	113	0.33
7	67	0.36
Mean	234.5	0.81

Table 3. Comparison of dry weight of organic matter of plankton in eutrophic lakes in Japan

Lake	Dry weight of plankton (mg/m³)	(kg/ha)	Weight of organic matter of plankton (kg/ha)	Investigator
Suwa	11500–2500		336–24	Hogetsu ***et al.***, 1952
Nakanuma	1330–840			Ichimura, 1956
Koikuchinoike	881	125.4	69.0	Kawamura, 1956
Nukabira	234.5	561.3	252.6	Kawamura & Anraku, 1956

Several authors (Hogetsu *et al.*, 1952; Rawson, 1953; Ichimura, 1956; Kawamura, 1956) gave figures on the measurement of plankton standing crops in lakes; the volume was 10–20 kg in dry weight per hectare in oligotrophic lakes (western Canada), 50–100 kg per hectare in moderate lakes, about 150 kg per hectare in very eutrophic lakes. The production of the present lake which yields a crop of 234.5 mg per cubic meter or 561.3 kg per hectare seems to rank as a lake of eutrophic type, though the value here reported is concerned only with the time of observation, not annual mean.

The amount of chlorophyll of plankton in the present lake ranged from 0.1 mg to 2.2 mg per cubic meter through all stations. Local difference of chlorophyll content of plankton was generally parallel to that of the dry weight of plankton. Ichimura (1956) stated that on the basis of summer observations, the eutrophic lakes such as Lake Suwa, Lake Nakanuma, *etc.* contain plankton chlorophyll in the range from 10 mg to 50 mg per cubic meter of water, while in the oligotrophic lakes such as Lake Aoki, Lake Motosu, *etc.* it is less than 1 mg per cubic meter. Other lakes showing intermediate values between them indicates that they are mesotrophic. From this classification, the present lake is assumed to be of oligotrophic type. It is general fact that the amount of chlorophyll of plankton in a lake varies greatly from season to season, so that the annual mean of chlorophyll content in this lake may be larger than the above estimation (0.81 mg/m^3) obtained in November. The present lake will possibly come within the range of the mesotrophic lakes at the present time.

Summary

1) A preliminary limnolgical survey of Lake Nukabira, a body of fresh water impounded as a reservoir about one year previously, was carried out in November, 1956.

2) The character of a bog lake in respect to physical and chemical properties was not recognized. The plankton species are confined to 8, among which *Bosmina coregoni* and *Asterionella formosa* are dominant.

3) Such nutrient substances for the growth of phytoplankton as silicate-Si and phosphate-P in the lake water are remarkably reduced by the vigorous growth of the diatom, *Asterionella formosa.*

4) The dry weight of plankton is calculated to be 561.3 kg per hectare, indicating that the lake is eutrophic to a certain extent, but the quantity of plankton chlorophyll is rather low, corresponding to that of usual oligotrophic lakes.

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DIURNAL RHYTHM OF THE FEEDING ACTIVITY OF GOLDFISH IN WINTER AND EARLY SPRING

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From the view point of fish culture, it is an important problem to investigate the feeding activity with which fish begin to feed in such special seasons as winter and early spring. In such a meaning, it is worthy to mention that Kinoshita[1] has discussed the existence of correlation between the initiation of the feeding behaviour in early spring and the environmental water temperature in wrasse-fish. Krayuhin[2] has reported also that the feeding-latent period in carp varies considerably corresponding to change either rise or fall in the water temperature. Additional to these works, some careful studies have been reported with the purpose of rationalizing the various problems in fish-culture.[3-6]

The writer has reported his studies made concerning the influence of the environmental factors on the pattern of the feeding activity of goldfish in autumn and early winter, using an automatic fish feeding apparatus.[7] In the present paper, similar studies made in winter and early spring are described.

The writer wishes to thank Prof. S. Kobayashi of the Faculty of Fisheries, Hokkaido University, for his valuable advice rendered during the course of the present experiment and for his kindness in reading the original manuscripts. Thanks are due to the staff members of the Hakodate Marine Observatory for supplying the data of the climatic observations used in analysis of the experiment. The writer is also indebted to Miss N. Shimizu, who helped him to count the feeding number recorded on the kymograph.

MATERIAL AND METHOD

All the experiments were performed in a greenhouse of the Faculty of Fisheries; the kymographic records of the fish feeding were taken every day during the period from January 9 to April 2, 1956. The size of the tank used is approximately 140 cm long, 70 cm wide and 45 cm deep. The depth of the water was maintained at about 35 cm, a little well-water flowing into the tank. Throughout the present experiment, 30 goldfishes one year old were used. The average total body length of them was 52 mm at the outset of the experiment and 62 mm at the end.

The feeding activity was investigated using the feeding recorder as indicated in the preliminary report,[8] and the relative value of the said activity was judged by the feeding frequency recorded on the kymograph. That is, to obtain the feeding records automatically, a bait box was suspended about at the center of the tank of water to tempt the fish to peck it. Minced crustacean larvae were used as food throughout the present experiment. The bait was changed every day for the fresh material which had been proved

preliminarily in quantity sufficient for a day's ration. As already stated in the previous paper,[8] the disturbance of the feeding behaviour by the changing of the bait was negligible.

RESULTS

Diurnal rhythm of the feeding activity in winter and early spring

The daily frequency of the feeding was highly variable. So, to show the general appearance of the diurnal rhythm of the feeding activity, the average frequency per hour was calculated for every ten-day period of the months during which the observations were continued. The results are illustrated in Figs. 1 and 2. As is clear from these figures, the feeding activity in the period shown in Fig. 1 is much lower than that of Fig. 2. Therefore, from the ecological point of view, the writer called the period of the former case (Fig. 1) "winter" and that of the latter case (Fig. 2) "early spring" in the feeding activity of the goldfish in Hakodate.

As already reported in the previous paper,[8] the feeding activity of the goldfish in the seasons of the autumn and early winter was found to decrease gradually with days. However, in the period from winter to early spring, such activity was found to increase suddenly, especially, after the second decade of March. That is, until the last decade of February, the feeble daily feeding is almost restricted to a few hours from about the 13th hour to the 15th, in which time the water temperature was highest. Further,

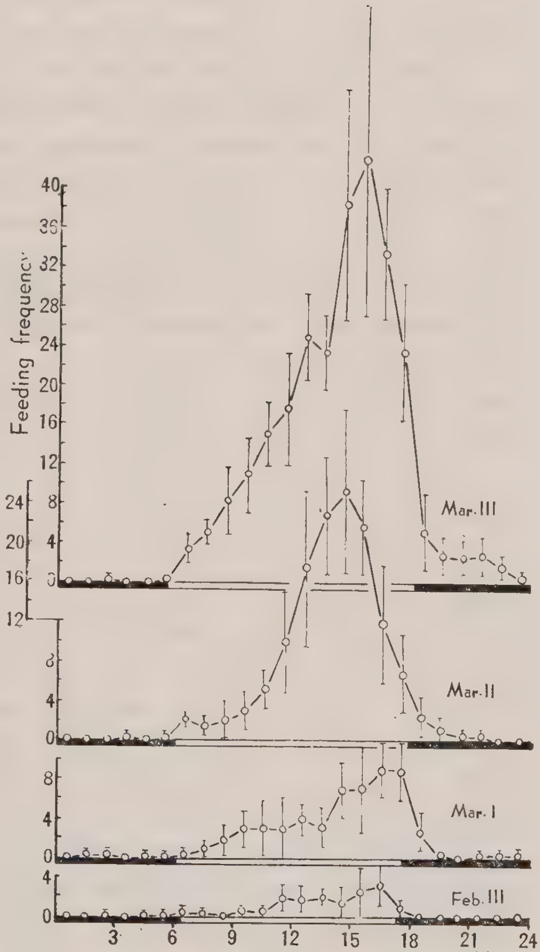


Fig. 2

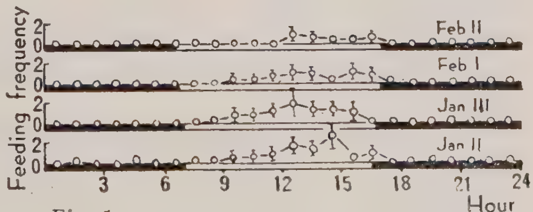


Fig. 1

Fig. 1 & 2. Time and diurnal rhythm of the feeding activity in the goldfish are shown in the average of every decade of days from January to February. The Roman numerals I, II and III mean the first, middle and last decade of every month, respectively. Each bar indicates the standard deviation of the feeding frequency.

the writer has considered that it is doubtful whether all the feeding records obtained in this special season are those which were marked by the actual feedings or only recorded by accidental touches of the fish body to the bait box. At any rate, it can be said that the feeding of the goldfish almost ceases in the "winter" season.

Then, with the passage of the season from winter to early spring the feeding of the fish becomes active suddenly, being highly accentuated after the second decade of March. Such a relationship will be seen also in Table 1.

Table 1. Daily fluctuation of the feeding frequency of the goldfish in the period extending from the first decade of January to the first decade of April. Number and the mark "+" mean that the recording was interrupted in the course of the experiment on that day.

Month	Decade \ Date	1	2	3	4	5	6	7	8	9	10	11
Jan.	I	—	—	—	—	—	—	—	—	7+	1	
	II	21	5	27	18	10	1	2	5	1	16	
	III	13	8	3	6	8	23	24	10	1	3	1
Feb.	I	4	8	15	16	1	2	3	1	1	2	
	II	0	0	0	1	0	0	6	3	11	5	
	III	30	25	15	17	18	21	12	5	10		
Mar.	I	24	39	36	28	31	48	56	40	160	75+	
	II	24	208	56	464	56	—	—	—	—	—	
	III	134	449	497	245	72	34	182	362	464	359	266
Apr.	I	111	13+	—	—	—	—	—	—	—	—	

The general appearance in the diurnal rhythm of the feeding activity in winter is slightly different from that of the autumn and early winter. In the autumn and early winter, the feeding begins one or two hours before sunrise, and reaches the peak in the time from about the 11th hour to the 15th. While in the period of winter and early spring the feeding begins a few hours after sunrise, and reaches the peak at the time of sunset, though the times of the peaks in the period of the middle and last decades of March are found to approach to those of the autumn and early winter.

Correlation between the feeding activity and the environmental factors

As stated in the former section, the feeding becomes active with the passage of the seasons from winter to early spring. Feeble feedings were found, even in the winter season, to occur in the period when the water temperature showed the highest degree. Considering from these facts only, it seems to be clear that the water temperature is one of the most important factors effecting the feeding activity, at least, in the seasons of winter and early spring.

Generally saying, in these seasons the feeding of the goldfish begins at the time of

sunrise and ends at the time of sunset, i. e., it is restricted to the daylight hours. Consequently, it is clear that the diurnal rhythm of the feeding is closely associated with light environment.

An analysis was made concerning the correlation between the feeding activity in these seasons and the other environmental factors, in addition to the factors of light and water temperature.

Feeding activity in winter

An example is given in Fig. 3 to show the correlation between the feeding activity and certain environmental factors. The feeding frequency in winter is very low though

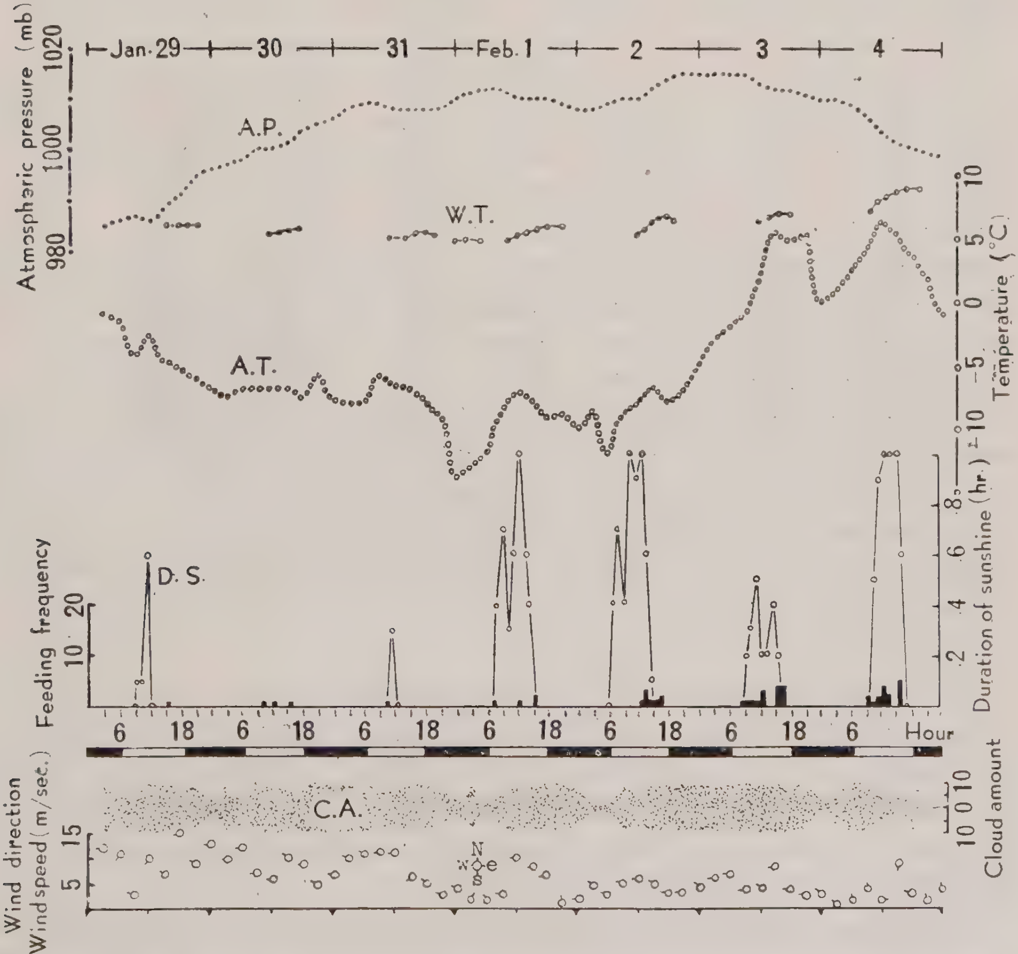


Fig. 3. An example of the diurnal rhythm of the feeding activity in the goldfish in winter, in relation with the environmental conditions. Environmental factors; o-o-o-o-o-o water temperature (W. T., C°); oooooooooo air temperature (A. T., C°), o——o duration of sunshine (D. S., hours); ••••••• atmospheric pressure (A. P., mb), cloud amount (C. A., every-hour index of 0–10); wind speed (m/sec.) and wind direction.

10 times or more were often recorded. During the days from January 29 to 31, only 1–3 times of the feeding were recorded. As a sharp depression of the atmospheric pressure occurred on January 28 and 29, that factor might have influenced the feeding activity of those days. According to the previous study,[8] the supernormal feeding was usually found to occur following the recovery of the depression of atmospheric pressure, if the other environmental factors are favorable for feeding. However, such a supernormal feeding was found neither on January 29 nor on the 30th. From these circumstances, it is considered that the feeding activity of these days decreased possibly owing to the occurrence of some other unfavorable environmental factors. The maximum water temperature during the days from January 29 to February 1 was below 6.5 °C. From February 2 onward, the water temperature begun to rise just a little, the maximum value of February 4 showing 8.4 °C. Even because of such a slight rise of the water temperature it appears that the fishes became a little more active. The assumption will be supported by the fact that 15 and 16 times of feeding were recorded on February 3 and 4 respectively. So, some combination of the environmental factors which bring about elevation of the air temperature is considered to exert an important influence upon the feeding in winter. Possibly, the gradient of atmospheric pressure and increases of both solar radiant energy and duration of sunshine may be cited as such factors. However, as is clear from Fig. 2, at least in the winter season, the water temperature does not always rise corresponding to increase of the duration of sunshine. Consequently, of the three factors cited above, the gradient of the atmospheric pressure may be a principal factor which encourages the active behaviour of the fish in the last phase of the winter season. The feeding activity of the goldfish in winter may be said to be severely inhibited or to be forced to the state of complete cessation by low water temperature, and the fishes appear to feed a very little only in the environmental conditions which are exceedingly favorable for their life in this special season.

Feeding activity in early spring

Fig. 4 presents an example of the correlation between the feeding activity in early spring and the environmental factors. The daily feedings within the three days from March 22 to 24 showed more than 245 times (cf. Table 1). The water temperature during these days was high, that of the daytime showing more than 9 °C. The duration of sunshine was 3.2, 7.8 and 10.5 hours respectively. The atmospheric pressure varied through these three days without any marked fluctuation.

The feeding activity was severely depressed on both March 25 and 26, though the water temperature during these days did not appear to be different from that of March 22, showing equally more than 9° C. The duration of sunshine was 0 and 0.8 hours respectively. The atmospheric pressure continued throughout these days with a relatively low value. A combination of the depressions of the atmospheric pressure, duration of

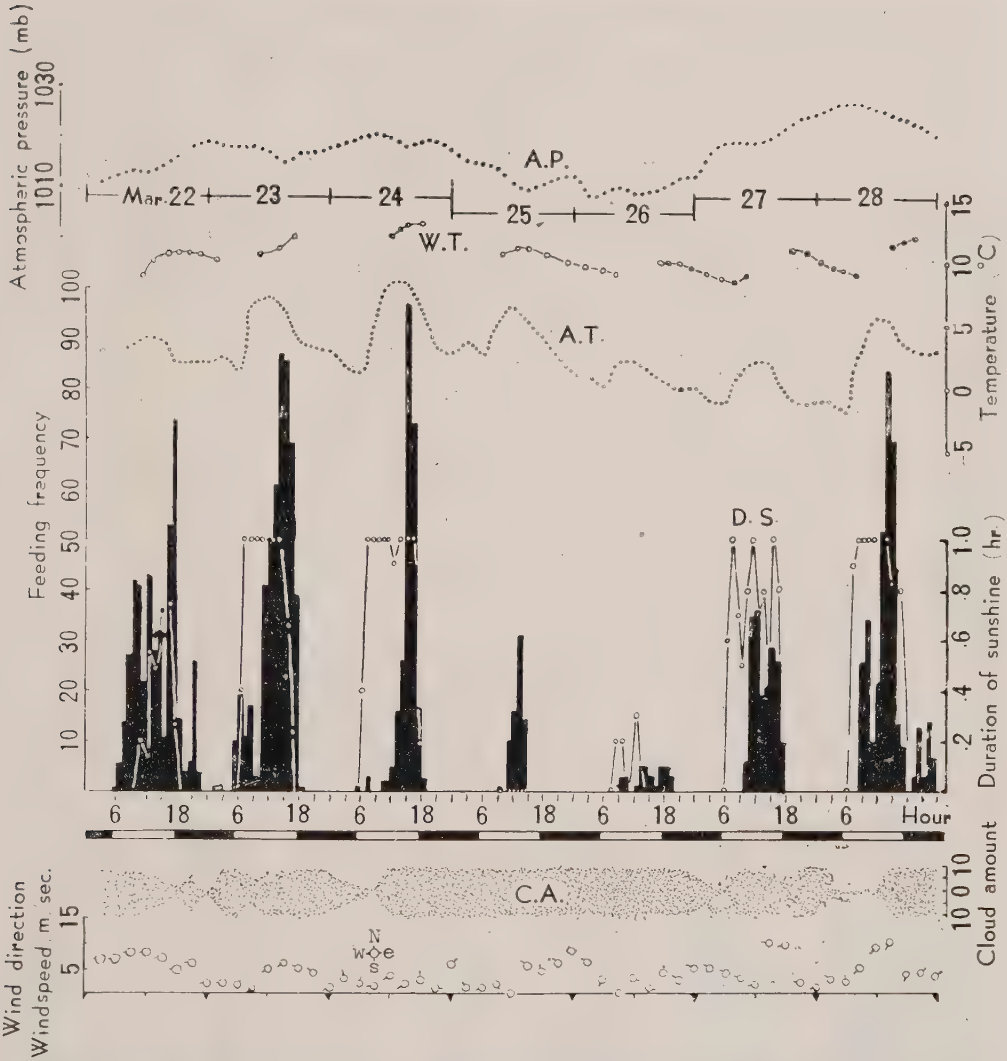


Fig. 4. An example of the diurnal rhythm of the feeding activity in the goldfish in early spring, in relation with the environmental conditions. Environmental factors; o-o-o-o-o-o water temperature (W. T., C°); oooo.ooooo air temperature (A. T., C°); o——o duration of sunshine (D. S., hours); ······· atmospheric pressure (A. P., mb); cloud amount (C. A., every-hour index of 0–10); wind speed (m/sec.) and wind direction.

sunshine and some other factors might have caused a lowering of the air temperature in comparison with that of the preceding days. Possibly, it seems that such a decline of the weather as a whole might bring about the depression of the feeding activity of these days.

Following the cloudy days of March 25 and 26, it was fine for several days consecutively. In company with such a recovery of the weather, the feeding frequency

increased very distinctly as shown in Fig. 3 and also in Table 1. On March 27 and 28, the water temperature in the daytime showed more than 11 °C. The duration of sunshine of those two days was 8.4 and 9.5 hours respectively. The atmospheric pressure remained steady with a high value throughout these days.

It is presumable from these facts that the feeding activity in the season of early spring or in the period when the said activity is in the state of increasing phase, may not be so severely distorted as either in the period of falling phase such as autumn and early winter or as in the winter season, even if the environmental factors change considerably,

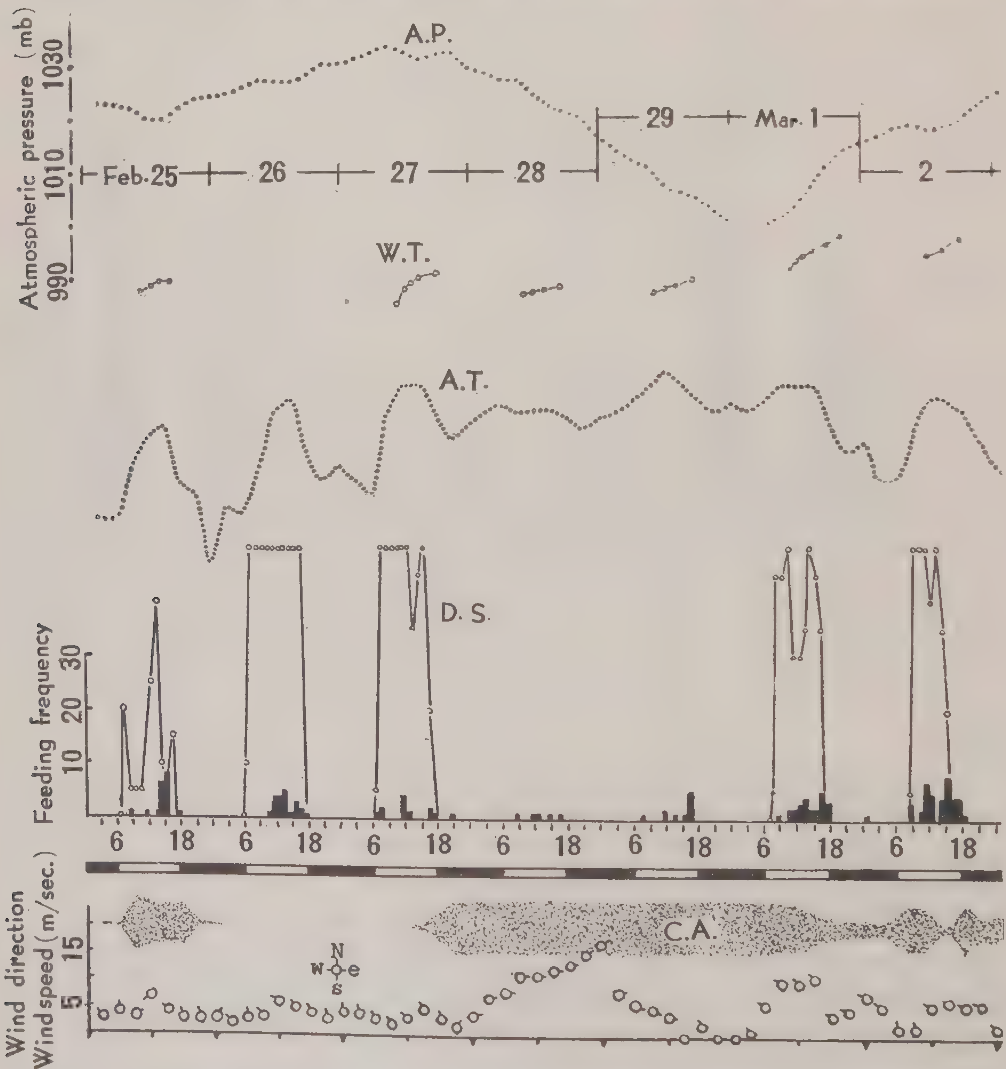


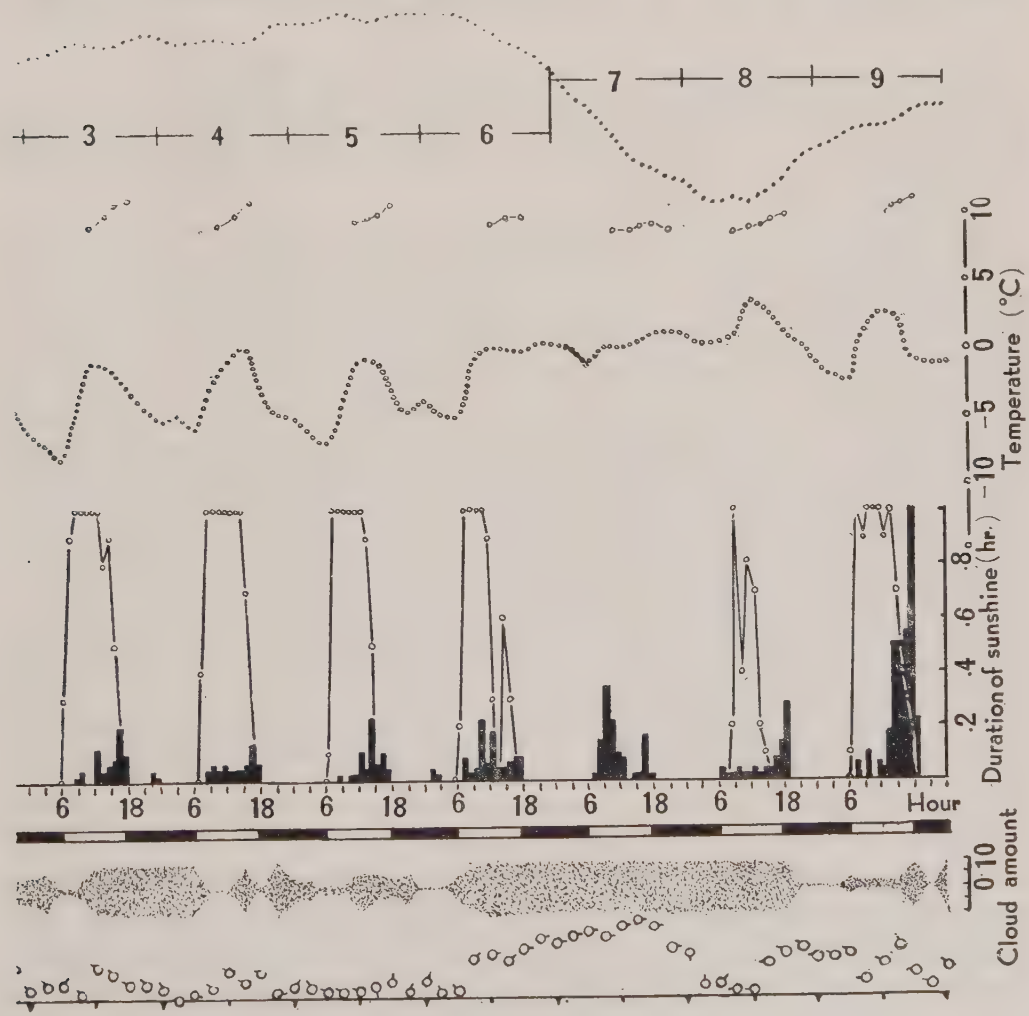
Fig. 5. An example of the diurnal rhythm of the feeding activity in the goldfish factors; o-o-o-o-o-o water temperature (W. T., C°); oooooooooo air temperature (A. T., C°); cloud amount (C.A., every-hour index of 0-10); wind speed (m/sec.) and wind

in case the inititiation of the feeding has once started vigorously.

Initiation of the feeding activity in early spring

In view of food-supply for fish, it is an important problem for the fish-culturists under what environmental conditions the goldfishes begin to feed in early spring waking from the long period of hibernation in winter.

As indicated in Table 1, until about February 20 the fishes almost did not feed. But, in the period from the last decade of February to the first decade of March, the daily feeding frequency increased gradually with the days within the approximate range



in winter and early spring, in relation with the environmental conditions. Environmental
o——o duration of sunshine (D. S., hours); ······· atmospheric pressure (A. P., mb);
direction.

of 20 to 30 times. Following such a transitional period of activity, the distinct recovery was found to occur with the daily frequency more than 50 times. Thereafter, it was not rare that the daily feedings are beyond a few huudreds.

Fig. 5 shows the correlation between such a transitional pattern of the feeding activity and the environmental factors. In the period from February 25 to March 1, 5–24 times of feeding were recorded. The water temperature of the daytime during this period varied within the range of about 7–9 °C, except for March 1 on which day the maximum water temprature showed as high as 12 °C. Such a temperature as 12 °C was the highest degree during the neighbouring days. Yet, the feeding frequency of this day was small in number, recorded as only 24 times.

The decrease of the feeding activty on both February 28 and 29 might mainly be caused by the bad weather such as the sharp depression of the atmospheric pressure from about 1028 to 1000 mb and the lowering of the duration of sunshine which showed almost zero on both days.

In the period from March 2 to 8, the feeding activity increased gradually, and on March 9 the said activity rose up suddenly being recorded as 160 times. Analyzing the environmental factors which may have induced such increase of the said activity, every one will first notice the fact that the fine days continued from March 2 to 6. The maximum water temperature during these days was about 11°C. The duration of sunshine varied showing high values in the range of 6.0 – 8.4 hours. Meanwhile, in the afternoon of March 6, a snow storm came on, accompanied with a sharp depression of the atmospheric pressure from 1032 to 997 mb and also with the lowering of the duration of sunshine to zero on March 7. However, the feeding activity was not depressed on these days; as already reported in the previous paper the said activity in all cases, at least, in those studied in autumn was severely distorted by such a sharp depression of the atmospheric pressure. Consequently, at first glance, this case appears to be somewhat exceptional. But, it will be recognizable from Fig. 5 that in the period of the snow storm the air temperature rose to 0°C, possibly resulting from the change of the wind direction. Such a compensatory factor might prevent depression of the feeding activity which would bo expected to occur.

A high feeding activity was recorded on March 9. This may be said to be a distinct recovery of the feeding from the hibernation period of the fish. Possibly, such a recovery might mainly be resulted from some favorable environmental conditions such as the fine weather of the consecutive five days from March 2 to 6. So, if the bad weather of March 7 had not occurred, recovery of the feeding activity might have occurred earlier, for example, on March 7 or 8. Considering from these facts and also from the assumption described above, it seems that recovery of the feeding activity of the goldfish occurs following good weather of several consecutive days, under the condition of a water

temperature higher than 10°C.

DISCUSSION

Recently, Kariya[4] emphasized the importance of study of the feeding activity in fish culture. The writer has stated in his preceding paper[8] that the studies on the feeding rhythm in fish are important from the view point of food-supply for all fish-culturists. Although the writer is now working on the perfection of an automatic recording apparatus available for field investigations, even the apparatus used in the present experiment was useful to analyze the approximate tendency of the feeding activity in relation to the environmental factors.

It is considered that as the goldfishes in the tank of water are surrounded by many factors such as micro-organisms, chemical solutions, physical forces and so on,[9)-11] the diurnal rhythm of the feeding activity also is influenced by them.[6,12] Accordingly, relationship between the said activity in the goldfish and the environmental factors must be studied in detail in future. However, in agreement with the results of Spoor's experiment in goldfish,[6] it can be said from the present results that the feeding activity in the goldfish shows a distinct diurnal rhythm, and also that an annual variation of the said activity is clearly recognizable. The present finding seems to be noteworthy from the fish cultural point of view that the young goldfish waking from the winter hibernation begins to feed in early spring when the water temperature rose to more than 10°C. However, besides the circumstances described above, it appears to be essential for the initiation of the feeding in early spring that good weather continues for several days. It was clarified that the water temperature for encouraging the initiation of the feeding of the goldfish in early spring is a little higher than 8.5°C at which temperature they cease to feed in early winter. With regard to fish feeding in relation to the water temperature, Krayuhin[2] has found that feeding-latent period at the water temperature of rising from 4° to 10°C was longer than that of falling from 10° to 4°C, in the experiment of carp one year old. Kinoshita[1] has stated in his paper that in wrasse-fish the feeding initiates at a temperature higher than that at which they begin to hibernate. Therefore, the present results are in agreement with the findings of the previous workers who have studied on other species of fish.

In both seasons of winter and early spring it was ascertained that the water temperature is the most important factor controlling the feeding activity of the goldfish. The air temperature, solar radiant heat and duration of sunshine are considered respectively to be factors of the temperature environment. However, it appears that so far as the seasons of winter and early spring are concerned, the air temperature is the major factor in the temperature environment. In such a meaning, it is presumable that measurement of the gradient of the atmospheric pressure, the wind direction and some other factors

may produce change of the air temperature environment is necessary in any analysis of the correlation between the feeding activity in fish and the environmental factors. Having such a purpose in mind. investigation in this field is now in progress.

SUMMARY

1. Using an automatic recording apparatus, the feeding activity of the goldfish was studied in the period from winter to early spring.

2. It was clearly found that the feeding rhythm in these seasons is slightly different from that of the autumn and early winter already reported in the preceding paper. That is, in winter, the very feeble feedings are found to be restricted definitely to the time of the beginning of the afternoon at which time the water temperature showed the highest degree. In early spring, the said activity rises suddenly with a marked increase of the feeding. In such a period, the feeding begins after sunrise, reaches the peak from about the 13th hour to the 18th, and ends one or two hours after sunset.

3. In the period of winter and early spring, the water temperatue appears to be the most important factor which influences the feeding activity. In winter, even if the duration of sunshine is apparently favorable for feeding, it was not rare that only few feeding records were found. So, it may be presumable that in winter and also in early spring the air temperature is a main factor which causes change of the water temperature. For analysis of the correlation between the feeding activity in fish and the environmental factors, it seems to be also necessary to study such causes of change in the air temperature environment as gradient of the atmospheric pressure and wind direction.

4. Initiation of the feeding of the goldfish in early spring occurs on some days after fairly fine weather has continued for several days under the condition of the water temperature being higher than 10°C. Preceding such recovery, a transitional period of the feeding was found to occur under the conditions such as that the environmental factors, especially, the water temperature shows a distinct increasing phase. It appears that in such an improving phase of the environmental conditions the feeding activity is not so severely distorted by any unfavorable environmental factors as in the case of falling phase of feeding activity.

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青森県太平洋海区に於ける二隻曳(両手廻し)地曳網の研究

第1報　鯖鰮兼用二隻曳地曳網の構成に就いて

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Studies on the Beach Seines of Two-Boat Operation along the Pacific Coast of Aomori Prefecture

1. On the construction of the beach seine of two-boat operation used for both mackerel and sardine fishing

Giichi KAWASAKI, Sakuzo NISHIYAMA and Hideo NAKAMURA

Abstract

There are about 80 beach seines of two-boat operation in use along the Pacific coast of Aomori prefecture. The authors sampled 15 nets from them and made field studies on the construction of them, especially investigated the values of twine number, mesh size, length of finished nets, reefing (hopping), one-side length of finished wing net and bag net, wing height and circumference of bag net mouth.

The mean values and the standard deviations of them were calculate, and thereby the population means of these parts of the nets could be made clear.

The results are as follows,

1. On twine number
   Large mesh net : m = 4.9 ± 0.86　No. 4 wing net : m = 4.6 ± 0.73
   No. 3 wing net : m = 4.7 ± 0.92　No. 2 wing net : m = 3.7 ± 0.6
   No. 1 wing net : m = 3.4 ± 0.54　Bag net : m = 5.4 ± 0.75
2. On mesh size
   Large mesh net : m = 2.5 ± 0.46 (sun)　No. 4 wing net : m = 1.7 ± 0.38
   No. 3 wing net : m = 1.4 ± 0.38　No. 2 wing net : m = 1.0 ± 0.2
   No. 1 wing net : m = 0.85 ± 0.18　Bag net : m = 0.61 ± 0.09
3. On reefing
   Large mesh net : m = 0.2 ± 0.039　No. 4 wing net : m = 0.2 ± 0.043
   No. 3 wing net : m = 0.2 ± 0.039　No. 2 wing net : m = 0.2 ± 0.042
   No. 1 wing net : m = 0.19 ± 0.039 Bag net : m = 0.22 ± 0.039
4. On lengths of finished nets
   Large mesh net : m = 37.8 ± 10.1 (ken)　No. 4 wing net : m = 41.5 ± 10.2
   No. 3 wing net : m = 44.1 ± 11.8　No. 2 wing net : m = 34.1 ± 7.7
   No. 1 wing net : m = 30.0 ± 6.03　Bag net : m = 16.6 ± 3.0
5. On one-side length of finished wing net
   m = 187.3 ± 38.3 (ken)
6. On wing height
   m = 5.0 ± 0.69 (ken)
7. On circumference of net mouth
   m = 12.1 ± 1.4 (ken)

1 緒　　言

著者等は先に青森県水産資源開発調査が行われた際其の一部門として漁村実態調査の漁具の部を担当した。[1] 即ち津軽半島に属する東西全沿岸，青森北東夏泊半島野辺地湾を経て脇野沢に至る海区並に下北半島太平洋海区，尻屋岬より大畑に至る海区と三海区に分け各種網漁具及び釣漁具に就き調査を行い其の中，特に鯖鰮兼用の二隻曳地曳網の使用統数が多い太平洋岸に着目し爾来今日まで其の構成に関して北海道海区の各種地曳網[2] と比較調査を続行して居たが此の度，之等調査資料を利用し統計上，当海区の兼用地曳網は勿論の事青森県下の全太平洋に於いて使用される兼用地曳網と云う一つの母集団が如何なる構成になつて居るかを検討して見た。

即ち袖網及び嚢網各局部の号数，目合，掛目，仕立上りの長さ，縮結の関係並に片袖網全長と嚢網長さの関係，袖丈と嚢口全周との関係に就き其の大綱を知る事が出来たので此処に報告すると共に之等の構成の大綱に基き当海区の鯖鰮兼用の標準型二隻曳地曳網の設計を為し各種地曳網設計上の一基準に為るべく併せて此処に報告す。

2 資料及び調査方法

前記青森県水産資源開発調査が行われた昭和23年7月下旬より三週間同県太平洋沿岸即ち，八戸市より北上し尻屋岬に至る海区中，八戸（鮫町）市川村，百石村，三川目，尾駮浜，泊，白糠，入口村，尻屋及び尻労村を歴訪調査せし時の資料（実態調査並に聞き込み調査）を基にし且調査資料不充分なる海区に就いては後日郵送依頼に依り資料を取り纒めたものである。

そもそも此等の地曳網は引網類中の地曳網類に属するものであり本県に於いては上北郡海区のものは徳川初期，宮城県より移行されたものらしく又下北郡海区のものは140～150年前より北海道噴火湾のものに習い使用されたと云われて居り全海区の之等の構造は北海道海区のものと大差がない様である。

其の中，資材及び各局部の構成が明瞭なる鯖鰮兼用の二隻曳地曳網中より抽出した無作為標本15統を，青森県全太平洋海区に於いて使用される兼用地曳網と云う一つの母集団（population）より抽出された標本（sample）とし之より各局部の母平均の95％の信頼限界を求め検討を加えた。[3]

3 調 査 結 果

即ち兼用二隻曳地曳網の構造を袖網部と嚢網部の二部に分割し且袖網の部は荒手網，4脇，3脇，2脇及び1脇の5部に分割検討を加えて見た。

第1表は標本として取扱つた15統の構造を示した。

一般に各種漁網の構成，規模は其の目的漁獲物の種類及び特徴，習性其の他漁場等の点を考慮せねばならぬ事であり此の点より考えて勿論，鯖鰮兼用地曳網と限定されると第1表の如く自然各局部の号数，目合，縮結等は或る範囲内の数値を示す事が考えられる。

今，袖網及び嚢網部の各局部の号数，目合の度数分布を第1図，第2図に示した。此等の図を見るに大体正規分布（normal distribution）をなし即ち，それぞれの平均値の附近の度数が多く平均値から離れる程，度数が少い傾向が見られる。

次ぎに各局部の平均からの散らばり具合を見る為，標準偏差（standard deviation）を求めた。

1. 号数に就いては平均からの散らばりが1脇，2脇が割合小さく且，最も細目の網地を使用して居り荒手網は散らばりが大きく嚢網は最も太目の網地を使用して居るのが目立つ。

2. 目合に就いての散らばりでは嚢網，1脇，2脇が小さく荒手網が最も大きく，且，全般的に見て荒手網が一番大きな目合で嚢網に行くに従い小目を使用して居る。

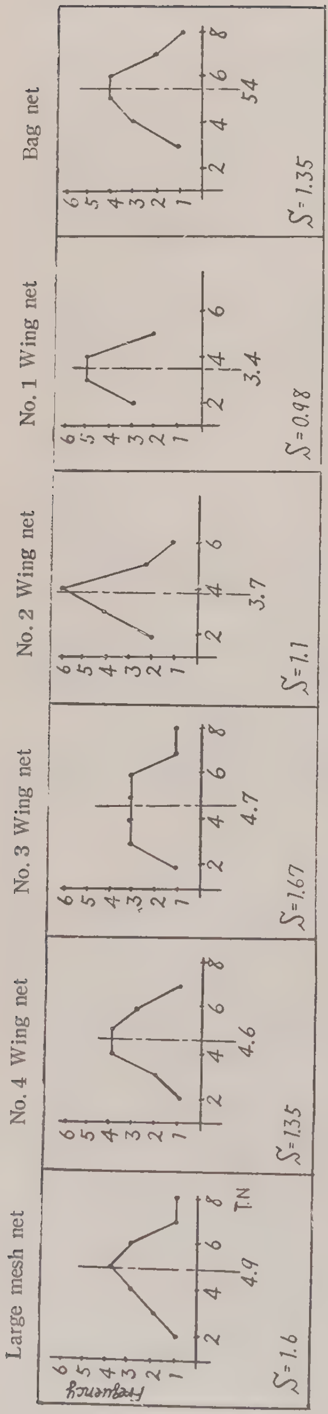


Fig. 1. Frequency polygon of twine number (T. N : twine number; S : standard deviation)

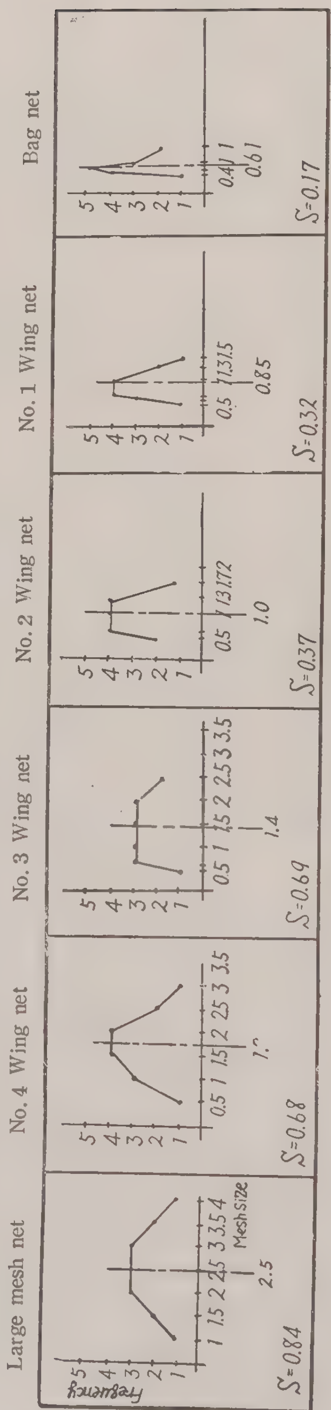


Fig. 2. Frequency polygon of mesh size

3. 縮結に就いて見るに15統の標本中，内割0.43と云う桁外れの縮結を多く入れた網があり此の点に就いて疑意が持たれたので一応荒手網に就いて Thompson の棄却検定法[4] を利用した。即ち，$F_0=7.6$，F-分布表で$n_1=1$，$n_2=13$，危険率5％に対するFの表値4.67であり内割0.43と云う標本は異常に飛び離れたものである事が解る。其の原因を当海区の資料或は北海道海区の各種地曳網の資料より検討して見たが各種地曳網の構成の点又は鯖鰮兼用二隻曳地曳網の構成から見ても斯様な縮結の多い網は構成そのものが間違つて居る事が解り標本の中より棄却する事にした。然る後，縮結の度数分布を第3図に示し平均値からの散らばり具合を見るに大差なく縮結は嚢網部に最も多く入れて居る。

4. 袖網の各局部及び嚢網部の仕立上りの長さに就いては各局部の長さが一様ではなく且，構成上長短の差が大きい為，袖網各局部に於いては20間以下，20～40間，40～60間，60～80間の4 classに分け又嚢網の部では10間以下，10～20間，20～30間の3 classに分け度数分布を第4図に示し且，平均からの散らばりを見るに嚢網，1脇，2脇等は特に小さく荒手網，4脇，3

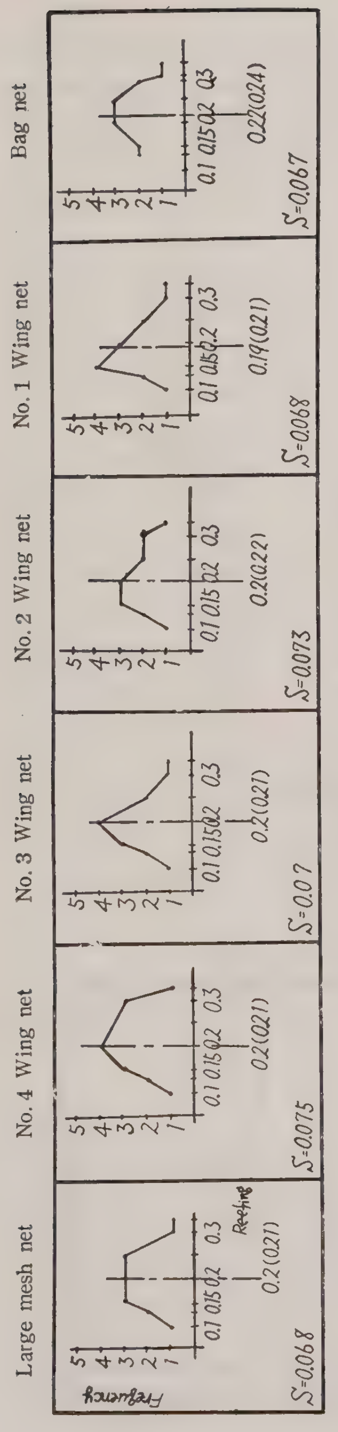


Fig. 3. Frequency polygon of reefing (Hopping)

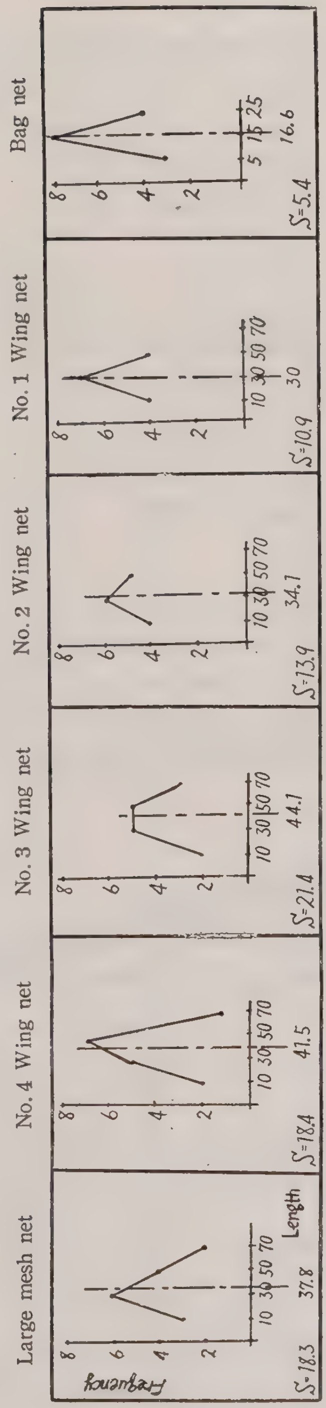


Fig. 4. Frequency polygon of finished wing and bag net length

脇は稍，同様である。又此等仕立上りの長さを縮結の点から見るに大体網地 100 間を囊網では 5 つ切り袖網では 2 つ半或は 2 つ切り位にして使用して居る事が解る。

5．片袖網地全長の仕立上りの長さに就いては50～120間，120～190間，190～260間，260～330間の 4 class に分け度数分布を第 5 図に示した。

6．袖網，囊網各部の掛数に就いて見るに之は勿論各漁場の水深による網丈の決定並に使用目合の決定で異るが荒手網の部に於いては目合別に見て大体，他の局部より掛目数に大差がなく 2 寸目では200目前後，2.5寸目，3 寸目では 2 寸目の時の80%前後の掛目数であり 4 脇の部では 1 寸目で450目前後又1.5寸目，2 寸目では 1 寸目の時の50%前後，3 脇の部では 6.3分目で 550目前後，1 寸目で450目前後，1.5寸目，2 寸目では 1 寸目の時の60%前後，2 脇の部では 6.3目で600目前後，1 寸目，1.3寸目では 6.3分目の時の70%前後，1 脇の部では 6.3分目，1 寸目では600～700目前後 1.3寸目では 之の50%前後，次ぎに囊網の部では 5 分目で2,400目前後，5.6分目で70%前後，6.3分目で65%前後の掛目数であつた。

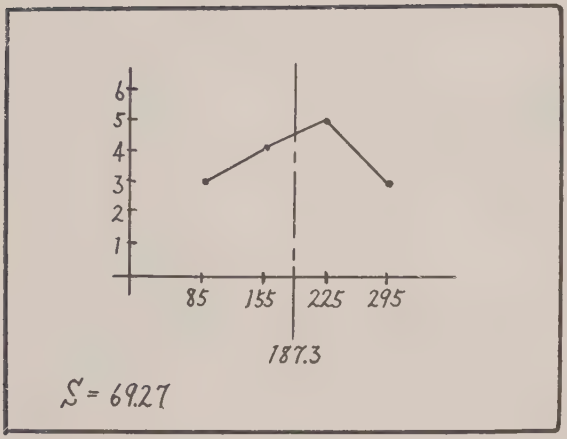


Fig. 5. Frequency polygon of one side length of finished wing net

7. 次ぎに網丈と嚢口全周との関係を見出すべく第2表に其等の数値を示した。即ち袖丈を2.5～3.5間，3.5～4.5間，4.5～5.5間，5.5～6.5間，6.5～7.5間の5 classに分け又嚢口全周を7～9間，9～11間，11～13間，13～15間，15～17間の5 classに分け此等の度数分布を第6図に示した。

Table 2. Wing height and circumference of net mouth (finished net length)

Sign of investigated net	Wing height	Circumference of net mouth
	ken	ken
A	4.1	9.4
B	6.5	14.6
C	4.9	11.7
D	7.3	16.6
E	6.7	15.7
F	5.4	13.0
G	4.3	11.6
H	3.0	7.5
I	4.6	13.0
J	4.3	11.0
K	5.7	13.0
L	5.0	11.0
M	3.9	10.0
N	6.1	14.0
O	3.5	9.0

Part of wing height　　　Part of net mouth

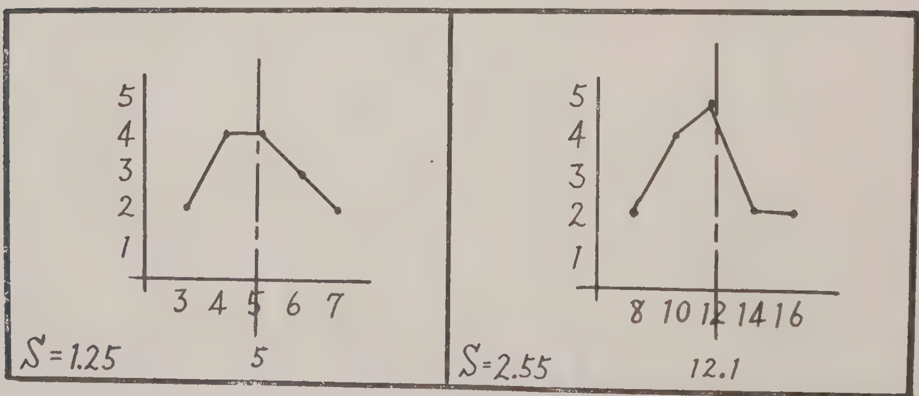


Fig. 6. Frequency polygon of wing height and circumference of net mouth

4 考 察

以上の各局部の関係より之等二隻曳兼用地曳網の標本を基にし青森県下の全太平洋岸に於いて使用される鯖鰮兼用地曳網と云う一母集団の各局部が如何なる構成になつて居るかを求むべく母集団平均値の推定を行い信頼係数95%の信頼限界（comfidence limit）を求めた。

即ち各局部の母集団平均をmとするとm$=\bar{x}\pm\frac{S}{\sqrt{n}}\sqrt{F^{1}_{n-1}}$の式より

1. 号数に就いては荒手網の部：m＝4.9±0.86（号），4脇部：m＝4.6±0.73（号），3脇部：m＝4.7±0.92（号），2脇部：m＝3.7±0.6（号），1脇部：m＝3.4±0.54（号），嚢部：m＝5.4±0.75（号）

2. 目合に就いては荒手網の部：m＝2.5±0.46（寸），4脇部：m＝1.7±0.38（寸），3脇部：m＝1.4±0.38（寸），2脇部：m＝1.0±0.2（寸），1脇部：m＝0.85±0.18（寸），嚢部：m＝0.61±0.09（寸）

3. 縮結に就いては荒手網の部：m＝0.2±0.039，4脇部：m＝0.2±0.043，3脇部：m＝0.2±0.039，2脇部：m＝0.2±0.042，1脇部：m＝0.19±0.039，嚢部：m＝0.22±0.039

4. 仕立上りの長さに就いては荒手網の部：m＝37.8±10.1（間），4脇部：m＝41.5±10.2（間），3脇部：m＝44.1±11.8（間），2脇部：m＝34.1±7.7（間），1脇部：m＝30.0±6.03（間），嚢部：m＝16.6±3.0（間）

5. 片袖網全長の仕立上りの長さに就いてはm＝187.3±38.3（間）

6. 袖丈に就いてはm＝5.0±0.69（間）

7. 嚢口全周に就いてはm＝12.1±1.4（間）

以上の構成に基き二隻曳鯖鰮地曳網の標準型の設計を為し第7図は一般完成図，第8図は網地の展開を示し此の構成は次ぎの如し。

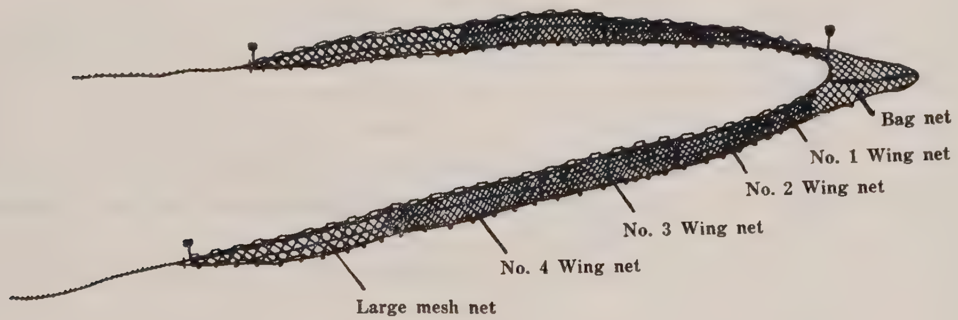


Fig. 7. Diagram of beach seine of two boat operation

荒手網（とで）

綿糸20番手4～5号2.5寸目，100目掛47.3間切（本目結節）横縫横目1.5反使用し尚半反の網地の一端より巾75目，長さ15間のさしわを切落し切落した方を沈子方とす。縮結は内0.2を入れ長さ浮子方37.8間，網丈手木に近い方を2.2間他方を4.5間に仕立上げる。

4脇部

同上綿糸1.7寸目，100目掛51.9間切横縫横目2.5反使用し縮結は内0.2を入れ浮子方41.5間網丈五間に仕立上げる。

3脇部

同上綿糸1.4寸目，100目掛55.1間切横縫横目3反使用し縮結は内0.2を入れ浮子方44.1間網丈5.1間に仕立上げる。

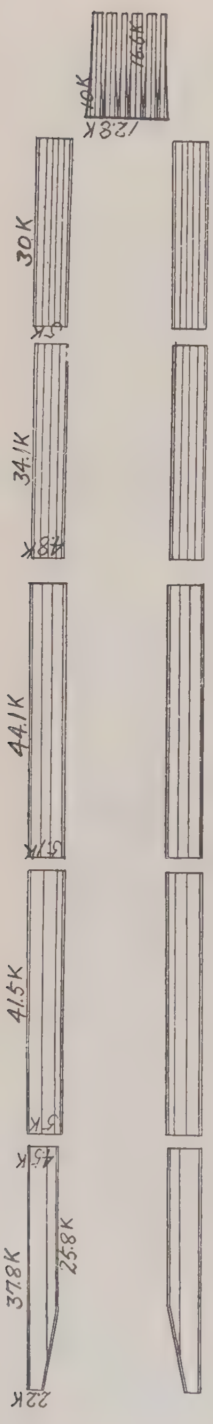


Fig. 8. Developed view of beach seine

2 脇部

綿糸20番手3～4号1寸目，100目掛42.6間切横縫横目4反使用し縮結は内0.2を入れ浮子方34.1間網丈4.8間に仕立上げる。

1 脇部

同上綿糸0.85寸目，100目掛37間切横縫横目5反使用し縮結は内0.19を入れ浮子方30間網丈5間に仕立上げる。

嚢網部

綿糸20番手4～6号0.61寸目，100目掛21.3間切横縫横目10反他に同上網地50目掛12.8間切の1反さしわ12枚使用し縮結は内0.22を入れ嚢口全周12.8間嚢長さ16.6間に仕立上げ且，之の網地を二つ折りに合せ接合部には「はつひやく」を用う。

縁網：浮子方は8号綿糸1.5寸目5目掛，沈子方は8号綿糸14節10目掛(嚢部を除く)

浮子網：マニラロープ3.5径，2分径の2本。

浮子方目通糸：岩糸2枚半又は30号綿糸を用い且，浮子網とは4寸間隔に絡む。

沈子網：マニラロープ3分径2本。

浮子：桐材9×3×1.5寸，間隔は浮子返しとす。

沈子：鉛50匁，1間に3～4箇の割合。

立木：楢材2～2.5寸径，長さは「とで」の巾にて決定す。

股網：マニラロープ6分径，14～15間を二つ折りに用う。

曳網：マニラロープ7～8分径400間位。

浮標(どん樽)：1.6～1.7尺径，高さ1尺これを嚢口の上部及び両股網に附す。

5 結　言

以上の調査結果より鰤鰮を目的とする地曳網の設計並に構成に関して大綱を知る事が出来，今後之等兼用二隻曳地曳網は勿論各種地曳網の一参考事項と成り得れば幸甚である。

尚，今後更に数多くの各種地曳網の資料を入手し精細なる検討を加え度いと思う。

6 文　献

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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

VI. Influence of the contained substances upon the growth of the formed $MgNH_4PO_4 \cdot 6H_2O$ crystals in test tubes

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It was stated in the previous paper[1] that the growth of the struvite in the canned sea foods will be influenced by the various substances contained therein. If the juice of the canned food is an approximate true solution such as pickle in the canned crab, numerous crystals will be rapidly formed in the can and will grow to larger ones. But, if juice in the canned food is a colloidal solution such as the juice in canned saury in which the skin of the fish is changed to gelatine by the processing, fewer crystals having smaller shape will grow in proportion to the viscosity of the juice.

The authors have studied how the crystallizing and the growth of the struvite in test tubes will be influenced by the contained substances.

1. Influence of the addition of gelatine

1) Experimental method

Two-one hundredth Mol magnesia mixture and 0.02 Mol sodium phosphate solutions were prepared as well as in the previous paper[2]. After 7.5 *cc* of sodium phosphate solution (0.02 Mol) was poured into each test tube, various amounts of gelatine were added and heated to dissolve in a water bath kept at 60 °C. Seven and five-tenths cc of magnesia mixture solution of 0.02 Mol were added to the above stated sodium phosphate solution, and thus solutions containing gelatine in the ratio of 5, 3.3, 1.7, 1.0 and 0.67% respectively were prepared. Those solutions were heated at 100 °C for 20 min. The solutions were cooled to room temperature. The formed crystals were observed under a microscope or by naked eye.

2) Experimental results

Results obtained are shown in Table 1.

As seen in Table 1, the larger the amount of gelatine contained in the solution, the more remarkably are influenced the velocity of crystallization and the velocity of growth of the crystals. In the mixed solution of the chemical constituents which was heated and stored for 50 minutes, when the solution contains 5~1.7% of gelatine, it becomes gel, and when the solution contains 1% of gelatine, many crystals were already formed. The size of the formed crystals in the solution containing gelatine is generally smaller than that of those formed in the solution without gelatine. When if gelatine was plentifully added to the mixture, after 50 days the crystals having no regular faces grew to

Table 1. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of gelatine

Leaving time \ Concentration of gelatine (%)		5	3.3	1.7	1.0	0.67
50 mins.	form	E	E	E	E	E
	size (μ)	—	—	—	—	—
14 days	form	—	—	C	C	C
	size (μ)	—	—	800×300	1000×300	200×60
50 days	form	C E	C E	D E	D	C
	size (μ)	51×5	25×20	380×1700	123～510 × 410～1800	31×130～300

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crystal, D=columnar crystal separated, E=amorphous

become pretty large crystals. But, there were a few that were visible, and almost all of them were microscopic. This fact agreed with the earlier finding[1] that the canned fish meat containing fish skin such as mackerel, salmon and saury have microscopic crystals. After storing of the solution at 37 °C for 10～15 days, the visible crystals were formed in the solutions containing from 1.0 to 0.67% of the gelatine. At concentrations of the gelatine above that upper mentioned, smaller crystals having irregular shape appeared. Accordingly, it may be said that the smaller the amount of gelatine the solution contains, the more regular are the shapes of the crystals which are formed. However, when those crystals formed from the solution containing gelatine are compared with the crystals formed from the solution containing no gelatine, the shape of the former is seen to be more irregular than the latter. A few crystals formed from the latter are separated; many of them gathered to make large crystals.

2. Influence of the addition of glycerol

Glycerol is colorless and odorless, and it is miscible with water in any proportions. The authors have tried to employ glycerol to prepare solutions having high viscosity like colloidal solution and to estimate the influence upon the shape and size of the formed crystal in the solutions to which it was added.

1) *Experimental method*

Ten *cc* portions of 0.02 Mol sodium phosphate solution to which 5 and 1 *cc* of glycerol respectively were added, are poured into separate Petri dishes and then 10 *cc* of 0.02 Mol magnesia mixture solution was added. Those solutions are stored in a thermostat kept at 37 °C, and the shape and size of the crystals formed were observed at definite time intervals.

2) *Experimental results*

Table 2 shows the results.

Table 2. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of glycerol

Leaving time (hrs.) \ Concentration of glycerol (%)		20	4.8	0
0	form	D	D	C
	size (μ)	76×17	87×19	48×6.4
20	form	D	D	D
	size (μ)	305×77	120×53	96×34
50	form	D	D	D
	size (〃)	312×116	308×98	270×104
	number (per *cc*)	5000	367	112

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crysral, D=columnar crystal separated

As seen in Table 2, immediately after the solutions are mixed, the size of the formed crystals is not remarkably influenced by the solution having various concentrations of the glycerol.

However, after being stored for a long time, the influence of the concentration of glycerol upon the size and the shape of the crystal appeared remarkably. That is to say, a few crystals of large size having regular surface were generated in the higher concentration of the solution. On the other hand, comparatively more crystals of small size having irregular surface appeared in the more dilute solution of glycerol. Those results may be explained by stating that when two solutions containing no glycerol are mixed, the components of the two solutions react immediately and form the crystals.

However, when the solutions containing glycerol are mixed, the reaction between the components in the two solutions will not occur completely, but only partially, and ionic components which have not reacted yet in the solution are gathered to react around the nucleus of the crystal formed at first; resultant larger crystals in smaller number are formed. In this case, the stabilization of the supersaturated solution in the colloidal solutions will also be sufficiently considered in the case of the formation of nuclear crystals.

3. Influence of the addition of agar

The authors have observed the formation of the crystal when agar was added to the mixed solutions of various concentrations.

1) *Experimental method*

Each 10 cc portions of sodium phosphate solution of 0.02 Mol were poured separately

into test tubes, to which 0.3, 0.2, 0.1, 0.04 and 0.02 *g* of agar were respectively added. The test tubes were heated at 70°C.

Ten *cc* portions of magnesia mixture were respectively mixed into the above described test tubes. In those test tubes, agar is contained respectively to the amount of 1.5, 1.0, 0.5, 0.2 and 0.1%. Those test tubes were plugged and stored at 37 °C. The changes in shape and size of the crystals formed in those test tubes were observed at definite intervals of time.

2) *Experimental results*

Results obtained are shown in Table 3.

Table 3. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of agar

Leaving time (days) \ Concentration of agar (%)		1.5	1.0	0.5	0.2	0.1	0
2	form	C	C	C E	C E	C E	C
	size (μ)	467×88	168×34	71×30	71×37	26×19	88×38
4	form	C	C	C	C E	C E	C
	size (″)	545×152	240×88	220×76	175×82	158×76	218×79
13	form	C	C	C	C E	C E	C
	size (μ)	1200×150	460×88	370×100	220×79	214×75	260×79

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crystal, D=columnar crystal separated, E=amorphous

As seen in Table 3, the condition of growth of crystals formed, the number and the shape of the crystals showed some differences with the concentration of agar added. The higher the concentration of agar was, the less in number and the larger in size the crystals formed. The surface of the crystals was somewhat coarse. Those phenomena will be also explained by the statement made in the previous article of this paper.

4. Influence of the addition of edible oil

As stated above, the formation of the crystals, $MgNH_4PO_4 \cdot 6H_2O$, is considered to occur easily in the so-called true solution which has small quantity of various mixed matters in the juice of the canned foods. However, even in the canned salmon of which the juice has a somewhat larger amount of oil, the formation of the crystals has been observed. Here, the authors have examined the influence upon the formation of the crystals resulting from the addition of edible oil.

1) *Experimental method*

Oil in the canned salmon (silver salmon) was isolated from the juice in a separating funnel. This oil was used as edible oil.

Each 10 *cc* portions of sodium phospahte solution of 0.02 Mol was poured into test tubes and 1, 0.5 and 0.25 *cc* of the salmon oil were respectively added to those test tubes. They were heated at 70 °C. Ten *cc* of 0.02 Mol magnesia mixture were added respectively to those test tubes. Those mixed solutions have respectively contained 5, 2.5, 1.3% of oil. Those solutions were heated at 37 °C. The number, the size and the shape of the crystals formed were observed.

2) *Experimental results*

Results obtained are shown in Table 4.

Table 4. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of oil

Leaving time (days) \ Concentration of oil (%)		5	2.5	1.3
2	size and form (μ)	31 × 19 (C) 3 × 1 (E)	49 × 14 (C) 31 × 19 (E)	360 × 27 (C) 38 × 25 (E)
	appearance	white and muddy	white and muddy	white and muddy
6	size and form (μ)	3000 × 500 (D) 3 × 1 (E)	124 × 26 (C) 31 × 19 (E)	130 × 38 (C) 38 × 25 (E)
	appearance	few crystals were sticked to the wall of the vessel	formed crystals were gathered at the bottom of the vessel	crystals were gathered as the form of branches
13	size and form (μ)	3500 × 1000 (D) 3 × 1 (E)	149 × 27 (C) 31 × 19 (E)	150 × 38 (C) 38 × 25 (E)
	appearance	few crystals were sticked to the wall of the vessel	formed cryatals were gathered	crystals were gathered as the form of branches

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crystal, D=columnar crystal separated, E=amorphous

As seen in Table 4, in the solutions containing larger amount of oil, the formation of the crystals was slow and the crystals were smaller in size and fewer in number. However, the crystals formed grew gradually to larger shape attaching to the side wall or sinking to the bottom of the test tube.

On the contrary, in the solutions containing smaller amounts of oil, the formation of the crystals was rapid and the crystals were small, but numerous. The crystals formed grew more slowly than in the above mentioned case. The size of the crystal was small.

5. Influence of the addition of materials which decomposed substances from protein

In order to ascertain the influence of the viscosity of the solution upon the formation of the crystals of $MgNH_4PO_4 \cdot 6H_2O$, the authors have added gelatine, glycerol, agar and edible oil to the mixing solution described in the previous article in this paper. Here,

the authors have examined the influence of the addition of material which was decomposed substances from protein upon the formation of the crystal, $MgNH_4PO_4 \cdot 6H_2O$. As the decomposed substances from protein, pepton* of various concentrations was used.

1) *Experimental method*

Each 10 *cc* portions of sodium phosphate solution of 0.02 Mol were poured into test tubes to which 1, 0.2, 0.1, 0.04 and 0.02 *g* of pepton were respectively added. Those test tubes were heated at 70 °C and 1.0, 0.2, 0.1, 0.04 and 0.02 *g* of pepton was respectively dissolved in those test tubes. Then, each 10 *cc* of magnesia mixture of 0.02 Mol was mixed to each test tube. The concentrations of pepton in those test tubes were 5, 1, 0.5, 0.2, and 0.1%. The test tubes were plugged and stored at 37 °C. The changes in the size and shape of the crystals formed were observed.

2) *Experimental results*

Results obtained are shown in Table 5.

Table 5. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of pepton

Leaving time (days)	Concentration of pepton (%)	5.0	1.0	0.5	0.2	0.1
0	size and form (μ)	12×2(E)	26×12(E)	19×15(C) 4×3	19×20(C)	19×12(E)
	appearance	white and muddy	white and muddy	white and muddy	white and muddy	white and muddy
2	size and form (μ)	23×38(C) 54×38(E)	11×4.5(C) 50×26(E)	85×53(C) 38×26(E)	63×67(C)	76×59(C)
6	size and form (μ)	58×38(C) 35×29(E)	30×15(C) 180×100(E)	190×150(C) —— (D)	260×79(D) —— (C)	180×64(C)
	appearance	formed crystals were gathered at the bottom of the vessel	formed crystals were gathered at the bottom of the vessel	few crystals were sticked to the wall of the vessel	few crystals were sticked to the wall of the vessel	few crystals were sticked to the wall of the vessel
13	size and form (μ)	170×100(C)	31×19(C)	180×110(C)	270×70(D)	220×93(C)
	appearance	no change	no change	no change	no change	no change

Note : Signs in ( ) are the same as the other tables.

As seen in Table 5, the differences in the size of the crystals formed were observed according to the concentrations of the solution of pepton. In above 1% of the concentration of pepton, the more irregular and smaller crystals generated. The crystals have had no regular surfaces at initial period, but after two days they were observed to become to have crystalline surfaces. In below 0.5% of the concentration of pepton, after the

* Pepton used in this experiment was that customarily employed in bacteriological study, Terauchi pepton.

mixing of solutions, numerous minute crystals having regular surfaces generated immediately.

After the crystallization, the crystals obtained from the solution of the concentration below 0.5 % were observed being attached numerously to the side wall of the test tube. However, the crystals obtained from the solution of the concentration of pepton above 0.5 % showed the powder-like precipitates (this is a mass of gathered crystals which are recognized as microscopical crystals of 10 ~ 20 μ).

In the pepton solution, some gathered crystals were recognized in the course of the growth of crystal after the crystallization, differing from what occurred in the solutions of agar and gelatine. Those states of the crystals were observed in the pepton solution in the various concentrations. Those states were observed earlier in the higher concentration of pepton than in the lower concentration of pepton.

Discussion

From the relation between the viscosities of the juice of marine canned foods and the formation of the crystals of $MgNH_4PO_4 \cdot 6H_2O$, the authors have ascertained that in comparatively larger viscosity of the juice, crystals of smaller size (microscopical size) were found, and in smaller viscosity the crystals of the larger size were found. However, the relation between the juice in the canned food and the formation of the crystals can not be explained by the viscosity of the juice only. There are many factors influencing the formation of the crystals, *e. g.*, the difference of the amount of magnesium, phosphate and ammonium in the juice, the quantities of the juice, the components of the juice (decomposing products of fish meat protein), movement of the juice during the heating and cooling of canned food.

The fact that the size of the crystals is small in canned mackerel, saury and salmon having large viscosity of the juice of the content is considered to be due to large quantity of the juice and to the convection which occurs at the cooling of the canned foods. Thus the stirring of the juice at the time of convection makes the size of the crystals small. This fact agreed with the fact that when a mixture of magnesium, ammonium and phosphate is cooled by stirring, the size of the crystals becomes small as described in the previous paper.[3)]

Summary

Gelatine, glycerol, agar, edible oil and pepton were respectively added in order to ascertain the influence of the viscosity of mixed solution upon the formation of crystals. According to the results obtained, if the viscosity of the solution is large, the crystals will generate slowly, while in a solution of low viscosity, the crystals will generate rapidly. However, the growth of the crystals after their first formation is rapid in the high viscosity solution. After long storing period, there appear few, but large crystals. In the

low viscosity solution, the velocity of the growth is slow; the crystals remain of small size.

These results are the same as the results which ensue upon the addition of edible oil. Apart from the substances such as gelatine glycerol and agar, having colloidal protective characteristics if pepton having lower molecular weight than the gelatine is contained to an amount over 0.5%, the crystals formed are apparently floury, but are actually a gathering of small thick columnar crystals as revealed under microscope. If the pepton is contained to an amount less than 0.5%, many crystals attached to the wall of the vessel. The gathering of the crystals after the formation will occur irrespective of the concentration of the solution. In the solution having high concentration the crystals will be formed more rapidly than in the solution having low concentration. In the solution containing pepton, the crystal is small in case of high concentration, and is large in the low concentration, differing from the cases of the concentration of gelatine, glycerol, agar or edible oil.

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2) ———————— (1957). *Ibid.* 8 (1), 59—64.
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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

VII. The formation of the separated microscopical crystal and its incipient growth

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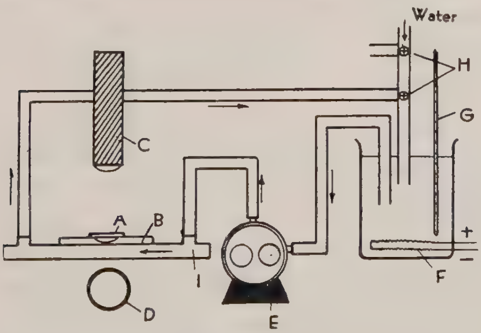
In the previous papers of this series,[1,2,3] the authors have reported investigations on the various conditions influencing the crystallization and the growth of crystals in Petri-dishes or test tubes. In those experiments, the state of crystals as they approached the final condition could be only observed. Therefore it was impossible to discuss all stages except only the final state of the crystals as they were formed in the test tube or Petri-dish. In this paper the authors record their investigation of various factors influencing the crystallization and the growing process of the same separated crystal under a microscope.

During the cooling process of the mixed solution, the velocity of the growth and the variation of the size of the crystals were observed under a microscope.

1. The velocity of growth of the crystals as influenced by the cooling velocity of the true solution

1) Experimental method

Each 0.01 Mol solutions of magnesia mixture (A solution) and sodium phosphate (B solution) were prepared as described in paper III[1]. Each 0.1 *cc* of B solution was taken onto a hole glass which was kept at 75°~80 °C by a small hot-water tank shown as Fig. 1, and then 0.1 *cc* of A solution was added and mixed.



Note: A = cover glass, B = hole glass, C = microscope, D = mirror, E = gear-pump, F = heater, G = thermometer, H = stop cock, I = small hot-water tank

Fig. 1. An apparatus for regulating the temperature of a hole glass

Next, water of various definite temperatures was made to circulate through the tank and the hole glass was kept at definite temperature. Thus the cooling velocity was changed at will. The condition of crystallization and the growing velocity at various velocities of cooling were observed.

2) Experimental results

At various cooling temperatures, the mean growing velocity of *c* axis and *a* axis indicated in Fig. 2 at the difference of

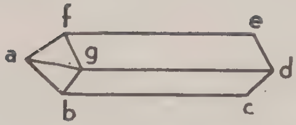


Fig. 2. Name of the crystalline axis
$\overline{bf} = \overline{ce} = a$ axis $\overline{ad} = c$ axis

10 °C, $\overline{L} = \frac{L_1 - L_o}{t}$ (μ/min.), in the cooling process was calculated from the growth of the crystal, $L_1 - L_o$; the results are shown as Table 1. Here, L_o is the initial length of the axis of the crystal; L_1, is the length of the axis of the grown crystal after t minutes; t is the time necessary to lower the temperature by 10 °C.

Table 1. Mean growing velocities of the crystals at various temperatures differing by 10 °C (μ/min.)

Temperature	Above 70° C	70°→60°	60°→50°	50°→40°	40°→30°	30°→20°
c axis ($\overline{ad}$)	3.25	3.14	2.48	5.35	1.85	0.91
a axis ($\overline{bf}$)	0	0.63	0.41	1.84	0.14	0.08

The cooling velocity is manifested by the equation: $\overline{\theta} = \Delta\theta / \Delta t$ (°C/min.).

Here, θ (°C) is the descending of temperature in t minutes. The relations between $\overline{\theta}$ and $\overline{L}$ are shown as Tables 1, 2 and Fig. 3.

As seen in Table 1, the mean growing velocity for the difference of 10 °C at various temperatures within the range of 20° ~ 70 °C at various temperature was maximum at the range of 40° ~ 50°C for c axis and a axis.

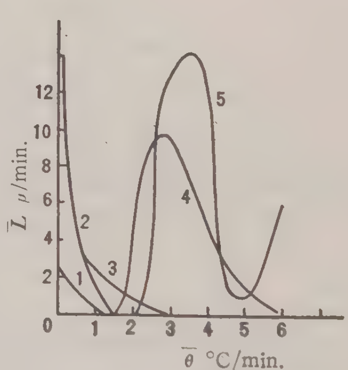


Fig. 3. Relation between the values of $\overline{\theta}$ and L

As seen in Table 2, when 0.01 Mol solutions each of A and B were mixed at 75° ~ 80 °C, and then the temperature of the mixed solution was made to fall, the amorphous minute crystals generated above 70° ~ 80 °C. But microcrystals (10~20×5~10μ) which can be found under a microscope by crystal surface and shape of the crystals generated at 60° ~ 70 °C. At temperatures below 50 °C visible crystals can be observed. This fact agreed with the result described in the previous paper, Report III[1], which was obtained in the combination of the component ions in test tubes. Especially, the crystals formed in the range of 50° ~ 30 °C are larger than those at other temperatures. This fact agreed with the thought of the authors to call this range the **"Zone of the formation of large crystals"**. The relation between the cooling velocity and the mean growing velocity varied with the range of the difference of the temperature. Above 60 °C, the relation was not remarkable, but in the range of 40° ~ 50 °C and 40° ~ 30 °C the relation was remarkably found. When the velocity of

cooling was small, the growing velocity of the crystals was found to be large.

Table 2. Relation between the cooling velocity ($\overline{\theta}$) and the mean growing velocity of the crystals ($\overline{L}$)

Range of temperature	Sample No.	Cooling velocity (°C/min.)	Mean growing velocity (μ/min.)	
			c axis	*a* axis
Above 70°C	Tr - 12	6.0	6.2	0
	Tr - 10	5.0	1.3	0
	Tr - 11	5.0	1.1	0
	Tr - 8	3.5	12.3	0
	Tr - 2	2.3	1.9	0
	Tr - 6	2.0	0	0
70° ~ 60°C	Tr - 12	5.9	0.65	—
	Tr - 11	5.0	1.1	—
	Tr - 8	2.6	9.4	—
	Tr - 9	2.3	5.5	1.8
	Tr - 2	2.3	3.2	0.6
	Tr - 6	2.0	0.6	—
	Tr - 5	1.7	0.8	0.4
	Tr - 1	1.5	3.2	0.65
60° ~ 50°C	Tr - 8	2.3	0.8	0.8
	Tr - 6	2.1	0.7	0.3
	Tr - 4	2.0	6.1	0.0
	Tr - 5	1.4	0.0	0.0
	Tr - 2	1.3	2.7	1.3
	Tr - 7	0.9	0.9	0.3
50° ~ 40°C	Tr - 5	1.0	0.7	0.0
	Tr - 7	0.7	4.0	0.3
	Tr - 2	0.6	1.9	0.7
	Tr - 8	0.5	12.0	0.7
	Tr - 12	0.01	8.2	0.9
40° ~ 30°C	Tr - 8	0.8	1.2	0.0
	Tr - 1	0.4	1.1	0.0
	Tr - 2	0.2	1.0	0.5
	Tr - 12	0.1	2.9	0.0
30° ~ 20°C	Tr - 8	0.8	0.7	0.2
	Tr - 1	0.3	0.6	0.0
	Tr - 5	0.2	0.9	0.0
	Tr - 12	0.1	0.3	0.0

2. The velocity of growth of the crystals in 0.01% pepton solution

1) Sample and experimental method

A and B solutions were prepared as stated above. Ten *cc* of B solution was taken into a test tube and pepton was added and heated at 60 °C to dissolve. Thus B′ solution was prepared to contain 0.02% of pepton. Following the same method as above stated, solutions A and B′ were mixed in equal quantities, the mean growing velocity L (μ/min.) was obtained for the range of the temperatures. The mixed solution of A and B′ contained 0.01% of pepton.

2) Experimental results

The mean growing velocities ($L=\frac{L_1-L_2}{t}$) for *c* axis in the respective range of the temperature for differences of 10 °C are shown in Table 3.

Table 3. Mean growing velocity of the crystals in the pepton solution (μ/min.)

Temperature	Above 70° C	70°→60°	60°→50°	50°→40°	40°→30°	30°→20°
c axis ($\overline{ad}$)	0.5	1.1	1.3	2.6	0.9	0.5
a axis ($\overline{bf}$)	0.3	0.3	0.3	0.5	0.4	0.2

As seen in Table 3, the mean growing velocity of the crystals is maximum in the range of 40° ~ 50 °C as was the case also in the true solution. However, the mean growing velocity was half of that in the true solution. This fact is considered to support the results reported in previous paper VI[4], that the crystallization is slow in solutions to which gelatine, pepton, agar had been added.

The velocity of growth of the crystal was reported as different according to the axis of the crystal, *i. e.* it is anisotropic, in the previous article. It was suggested that the growing velocity of *c* axis of the crystal is 5 times that of *a* axis in the true solution. However, in the pepton solution, the growing velocity of *c* axis of the crystal was about 3.5 times that of *a* axis.

In both solutions, long and thin pillar crystal (*triclinic system*) occurs, but the breadth is somewhat larger in the true solution than in the pepton solution.

3. The influence of the concentration of ammonium ion upon the formation of the crystals

In this article the influence of the ion concentration of ammonium upon the shape and the size of the crystals which were formed in the glass tubes or in the canned foods were observed.

1) Experimental method

Each 0.01 Mol solution of magnesia mixture (A′ solution) containing no ammonium

and sodium phosphate solution (B solution) was prepared according to the method described in paper III[1]. Also 0.056% ammonia solution (C solution) was prepared. As in the experiment above described, 0.1 *cc* of B solution was put onto a hole glass kept at 70° ~ 80 °C. Then 0.1 *cc* of a mixture of A′ solution and C solution (to 5 *cc* of A′ solution 0.1, 0.2 and 0.5 *cc* of C solution were respectively added) was poured onto the hole glass and the solutions were mixed in the hole. The shape and size of the crystals formed at a cooling velocity of 1°C/min. were observed.

2) *Experimental results*

Results obtained are shown in Table 4.

Table 4. Crystallizing state of the crystal of $MgNH_4PO_4 \cdot 6H_2O$ as influenced by the concentration of ammonia

Sample	Concentration of NH_4^+			States of the crystals
	In A′ solution	In C solution	In the mixed solution (A′. B. C.)	
1	0.0081 %	0.00055 %	0.0087 %	60×50 (μ) amorphous
2	0.0081 %	0.0011 %	0.0092 %	60×50 (〃) amorphous 34×9 (〃) crystals having surface
3	0.0081 %	0.0025 %	0.01 %	38×3 (〃) crystals having surface

As seen in Table 4, in the concentration of ammonium ion below 0.0092% crystals having regular shape did not occur, and only amorphous crystals generated. In other words, for the crystallization of the crystals having regular shape in the presence of 0.0122% of Mg, it was found that concentration above 0.0092% of ammonium ion is needed. When the concentration of ammonium ion is less than the equivalent amount of Mg, no crystal having regular shape generates. In this case, the molecule of NH_4Cl or NH_4OH was supposed to be entirely dissociated.

As Mg is contained in canned crab meat to the amount of about 0.01%, upon the basis of results above stated, then should be about 0.0092% of ammonium ion.

In the juice of canned crab meat 0.010~0.035% of ammonium is contained, so the crystals having regular shape will be formed as a matter of course.

Discussion

The crystallization of the crystal of $MgNH_4PO_4 \cdot 6H_2O$ is influenced by the concentration of the chemical constituents and the keeping temperature.

As seen in the second paper of this series[5], the solubility of the crystal was maximum at 50° ~ 65 °C, and that the solubility become less below or above a boundary of

50 °C. Therefore, the crystallization is considered to begin from 70° ~ 80 °C. The size of the crystal formed at 70° ~ 80 °C is too small to identify its shape and surface. The crystallization of micro-crystals having easily observable shape and surface occurs at 60° ~ 70 °C. But, the crystallization of visible crystals occurs below 50 °C.

In the process of cooling of canned foods, it is considered to be difficult to crystallize out at 70° ~ 80 °C because at that range of temperature the solubility of the crystal is small, and time required to pass the range of the temperatures is short, and the juice of canned foods contains high molecular substances (*e.g.* protein and its decomposed products). With the progress of the cooling process, small crystals formed at about 70 °C grow according to the decrease in the solubility.

The mean growing velocity for the range of 10 °C at various temperatures is remarkably large at the range of 30° and 50 °C. This agreed with **"Zone of the formation of large crystals"** described in paper III[1]. In this experiment, the formed crystals which were initially amorphous, were clearly demonstrated not to become large even after storing time. This fact is interesting, since it agrees with the result described in the previous paper[3] that is that the small crystals which have been formed by rapid cooling did not become large even if the storing conditions were good.

As important factors influencing the size of the formed crystals, there are the velocity of cooling at the time of the generation of the crystals at 70° ~ 80 °C and the ion concetration of ammonia.

Summary

Various factors exerting influence upon the crystallization and the growth of the crystals during the cooling process, *viz.*, the variation of the temperatures (cooling velocity) and the concentration of the mixed matters, were observed continuously and directly under a microscope.

In the process of cooling of the canned foods, the crystallization of the amorphous crystals usually begins at 70° ~ 80 C. This will be understood to occur because the solubility of the crystal is the minimum. The crystallizing temperature of the microcrystal (10~20×5~10μ) having regular surface and shape was found to be 60° ~ 70 °C. The mean growing velocity of the crystal was ascertained to be maximum at 50°~40°C and 40° ~ 30 °C according to the calculation for the range of 10 °C. This fact agreed with that the authors have called the range of 50 ~ 30 C **"Zone of the formation of large crystals"**.

According to the characteristics of the solution, the growing velocity of the crystal is different. The growing velocity in pepton solution is smaller than that in the true solution.

From the fact that the growing velocity is different from the crystalline axis, the

anisotropic character of the crystal was recognized. The ratio of the growing velocity in *c* axis to *a* axis was observed to be about 5. But it was 3.5 in the pepton solution. The relation between the cooling velocity ($\Delta\theta/\Delta t$) and the mean growing velocity of the crystal is kept constant in the range of 30° and 50 °C. If the velocity of cooling is small, the growing velocity of the crystal is large, and if the velocity of cooling is large, the growing velocity of the crystal is small.

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3) ―――――――― (1957). *Ibid.* 8 (1), 69—73.
4) ―――――――― (1957). *Ibid.* 8 (2), 115-122.
5) ―――――――― (1957). *Ibid.* 7 (4), 300-305.

THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

VIII. The formation of the crystals during the processing of the cans

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I. The amount of the chemical components, Mg and PO_4, which dissolved into boiling water

In the previous paper I[1], the amounts of magnesium and phosphate which were present in various kind of canned foods, were discussed. It is known with certainty that the process of boiling crab meat considerably influences the quality of the canned crab. For example, the quality of water used for boiling, or the boiling time influence the color and elasticity of the meat and the generation of blue meat of canned crab meat. The fact that water used for boiling has intimate relation to the crystallization of struvite in canned crab is understood from the experience that many pieces of struvite (visible or microscopical) generate in canned foods which were processed by sea water. In order to ascertain the amounts of Mg and PO_4 dissolved into the boiling water from the crab meat during the boiling process, the following experiments were performed.

1. Dissolving amounts of Mg and PO_4 in new and old boiling water used in preparation of crab meat

In many crab canneries, sea water is sucked up by a pump and is used for the boiling of crab meat. The removed crab leg meat with the crust from the carapace is heated in the sea water for 20 minutes. The same boiling water is used three or four times without changing. The water becomes dirty from the dissolving of the chemical components. The authors have estimated the difference of the amounts of Mg and PO_4 in the boiling water in accordance with the difference of number of times of use of the boiling water.

1) Experimental method

Each 200 *cc* of sea water which was newly sucked up and once used for the boiling and which was used successively three times for the boiling of crab meat, was taken, and 20 *cc* of nitric acid and 2 *cc* of hydrochloric acid were added and hydrolyzed. The hydrolyzates were diluted to 50 *cc*. Twenty cc of the diluted solution was changed to oxine compounds by the method described in paper II[2]. The amount of PO_4 was determined by the method described in American Official Agricultural Chemistry[3].

2) Experimental results

The amounts of Mg and PO_4 which were determined and compared in the water once

used or three times used for the boiling of crab meat are shown in Table 1.

As seen in Table 1, the greater the number of times of use is, the larger are the amounts of Mg and PO_4 dissolved into the boiling water.

Table 1. Dissolving amounts of Mg and P_2O_5 in new and old boiling water

	New boiling water	Old boiling water
The amounts of Mg (%)	0.00049	0.00079
The amounts of P_2O_5 (%)	0.00074	0.0019

2. The amounts of Mg and PO_4 dissolved in various kinds of water

The authors have determined the amounts of Mg and PO_4 dissolved in fresh water, sea water and various salts solutions used in the boiling of crab meat.

1) *Experimental method*

The leg with crust of crab which was caught off Nemuro in Hokkaido and removed from the carapace was brought in a thermos container to the laboratory. Ten gram portions of the leg meat were boiled in distilled water, sea water, N/10 $BaCl_2$ and N/10 $CaCl_2$ solutions respectively for 19 minutes. After the boiling, the amounts of Mg and PO_4 were determined in the filtered water from which the meat had been removed.

2) *Experimental results*

The results obtained by the above described proceeding are shown in Table 2.

Table 2. The amounts of Mg and P_2O_5 dissolved into various kinds of boiling water

Kinds of boiling water / Dissolving amounts (%)	Dist. water	Sea water	Solution of N/10 $BaCl_2$	Solution of N/10 $CaCl_2$
Mg	0.0028	0.0059	0.0028	0.0028
P_2O_5	0.0109	0.0075	0.0045	0.0033

Note: Amount of Mg in sea water was 0.0027.

As seen in Table 2, the amounts of Mg dissolved into various kinds of solution have no difference by the kind of water of solution used for the boiling. That is to say, the dissolved amount of Mg in every kind of water or solution was about 0.0028%. From the results described in paper I[1], the amout of Mg in crab meat has been known to be 0.106%. By the boiling of crab meat, the loss amount of Mg in the meat was known to be about 26%. The dissolved amount of PO_4 is different with the kind of water or solution for the boiling; the dissolved amount of phosphate was the maximum in distilled water.

The reason for the using of $BaCl_2$ or $CaCl_2$ solutions for the boiling was that

phosphate will be precipitated in the meat as Ca or Ba salts, and also to prevent the dissolving of phosphate into the solution. In fact, the amount of PO_4 in the $CaCl_2$ or $BaCl_2$ solutions used for the boiling was small for the reason stated above.

After the boiling in those kinds of water or solutions the quality and the color of crab meat showed no difference by the kinds of water or solutions.

3. The amounts of Mg and PO_4 in the crab meat after boiling in the various kinds of water and salt solutions

1) Experimental method

Crab meat boiled in various kinds of water and solution was washed once with distilled water and heated in separate Petri-dishes at 108.4 °C (5 *lbs/inch*^{2}) for 80 minutes. The heated dishes were cooled rapidly in running water; 6~7 *g*. of the heated meat was employed for the determination of the amounts of Mg and PO_4.

2) Experimental results

Results obtained are shown in Table 3.

Table 3. The amounts of Mg and P_2O_5 in the crab meat after boiling

Samples	Crab meat after boiling in			
	Dist. water	Sea water	N/10 $BaCl_2$ solution	N/10 $CaCl_2$ solution
Mg (%)	0.012	0.020	0.008	0.008
P_2O_5 (%)	0.050	——	0.079	0.093

As seen in Table 3, when sea water was used for the boiling, the amount of Mg in crab meat was maximum showing about 0.02%. This amount was about twice that in boiled meat heated in other kinds of water and salt solutions.

The amounts of Mg in the blocks of meat which were boiled in distilled water, $BaCl_2$ and $CaCl_2$ solutions were small. There is no difference between the amounts of Mg in meat which was boiled in distilled water and in $BaCl_2$ or $CaCl_2$ solutions.

4. Amounts of Mg and PO_4 in the separated juice from the crab meat boiled in various kinds of water and salt solutions

Determination was made of the amounts of Mg and PO_4 in the separated juice from crab meat heated in various kinds of water and salt solutions at 108.4 °C (5 *lbs/inch*^{2}) as described in the previous article.

1) Experimental method

A series of samples employed in Experiments 1, 2, 3, was used. Determination was made of the amounts of Mg and PO_4 in 2~5 *cc* of the samples of the separated juice obtained. The state of the crystals formed in the separated juice was observed.

2) *Experimental results*

Results obtained are shown in Table 4.

Table 4. The amounts of magnesium and phosphate in the juice separated from the boiled crab meat, and the form of the crystals formed in the juice

Sample	The juice which were separated from the boiled crab meat in			
	Dist. water	Sea water	$BaCl_2$ solution	$CaCl_2$ solution
Mg (%)	0.0062	0.012	0.0070	0.0020
P_2O_5 (%)	0.024	0.110	0.018	0.0060
Form of crystals	white flour-like	white flour-like	white flour-like	white flour-like

The amount of Mg in the separated juice from the crab meat boiled in sea water is larger than that in other various kinds of water. The amount of Mg in the crab meat thus boiled was about 0.012%. This amount was twice that in other kinds of water and salt solutions. However, there is no difference between the amounts in the boiling juice when distilled water and $BaCl_2$ were used. The crystals of $MgNH_4PO_4 \cdot 6H_2O$ in every separated juice were white minute flour-like.

Discussion

In order to learn about the transmission of the components of Mg which is a key point for the crystallization of $MgNH_4PO_4 \cdot 6H_2O$ and in order to prevent to the dissolution of the components of Mg and PO_4 into the juice separated during the boiling of crab meat by precipitating of the phosphoric acid as phosphate, various kinds of water and salt solutions were employed for the boiling. By the boiling of the meat in distilled water or $CaCl_2$ solution, the amounts of Mg in the separated juice decreased. The amount of Mg in the juice separated from the meat boiled in sea water is surely more than that in fresh water.

Even if the amount of Mg is small in the separated juice in the latter case, the amount is probably sufficient to form the crystals of $MgNH_4PO_4 \cdot 6H_2O$ as described in papers II[2], III[4] and V[5] of this series. In fact, crystals formed in the juice of the meat boiled in $BaCl_2$ and $CaCl_2$ solutions were white minute flour-like.

The color and the taste of crab meat which was boiled in fresh water became worse. Therefore it is undesirable to boil crab meat in fresh water. That is to say, it is practically better to use sea water rather than to use fresh water which is able passively to prevent the crystallization.

II. Relation between the cooling after the sterilization and the formation of the crystals

In the previous papers of this series[2)6)], the authors have reported their observations of the formation of magnesium-ammonium-phosphate crystals in test tubes and the growth of the separated crystals at various temperatures under microscope. The crystals are easily formed in ranges of temperature both above and below about 50°C as boundary. That is the maximum solubility of the crystal of $MgNH_4PO_4 \cdot 6H_2O$. In this case, above 50 °C amorphous crystals are formed, and below 50 °C crystals having easily observable shape occur.

The authors have observed the relation between the growth of the crystals and the velocity of cooling. In order to determine whether the relation holds in the canned food as well as in the test tube, or not, the authors have estimated the cooling curves after heating process of canned food according to the conditions of the cooling process, and have clarified the relation between the conditions of the cooling process and the formation of crystals.

1. Cooling curves of canned foods after heating process

1) Experimental method

Canned foods, crab (half pound, flat), salmon (half pound, flat), tuna in brine (half pound, flat), sardine (half pound, oval), after the heating process were cooled under the following various conditions.

i) Cooled in running city water in a vessel of 6～6.5 *l* —A-cooling method (rapid-cooling). This method is customarily employed in land canneries which have a large amount of water.

ii) Cooled by sprinking with city water in the amount of 6～6.5 *l* for 15～20 minutes. —B-cooling method (medium-cooling). This method is also used mostly in land canneries or on floating canneries.

iii) Cooled by air-blast by electric fun. —C-cooling method (slow-cooling). This method is usually employed in floating canneries which have only a small amount of water available.

The change of the temperature in canned foods during the cooling process was measured by a thermocouple.

2) Experimental results

As seen in Figs. 1～4, it is evident that the temperature at the center of various canned foods decreased logarithmically.

Here, for the purpose of comparing the cooling condition in the center of various canned foods which are cooled by C-method for example, the authors have estimated the value of the coefficient of the cooling velocity (n) according to the following equation. The obtained results are shown in Fig. 5 and Table 5.

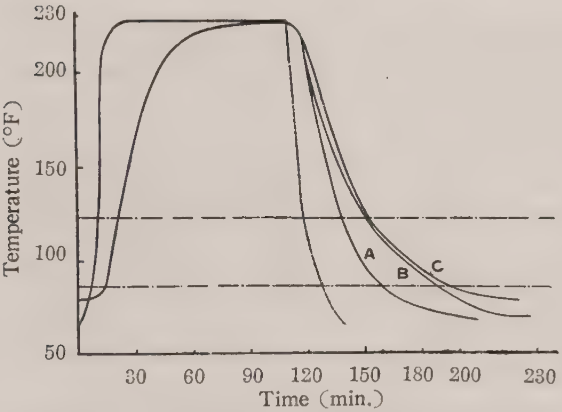


Fig. 1. Cooling curves of canned crab after heating process

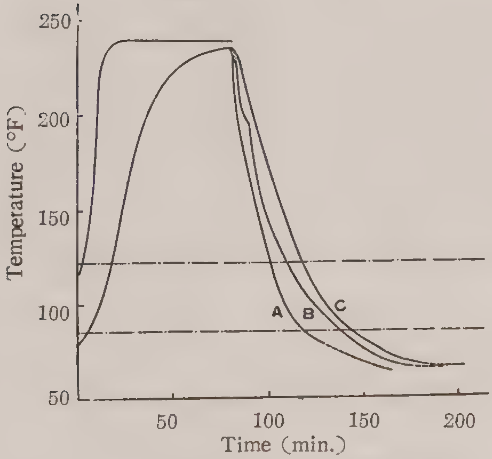


Fig. 2. Cooling curves of canned salmon after heating process

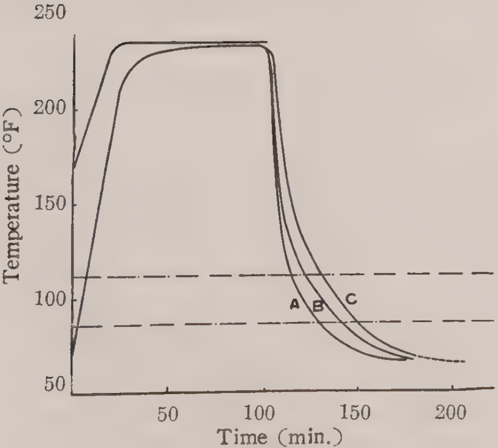


Fig. 3. Cooling curves of canned tuna after heating process

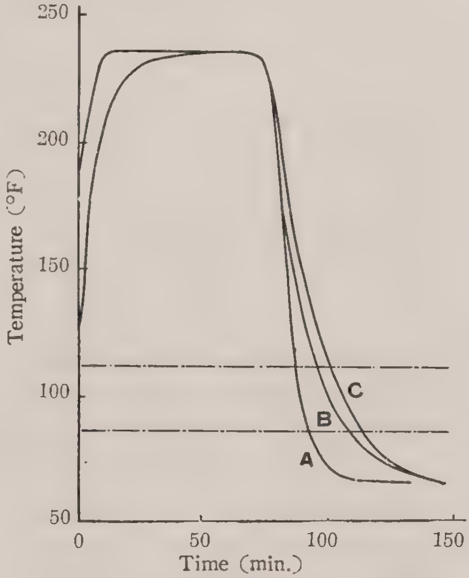


Fig. 4. Cooling curves of canned sardine after heating process

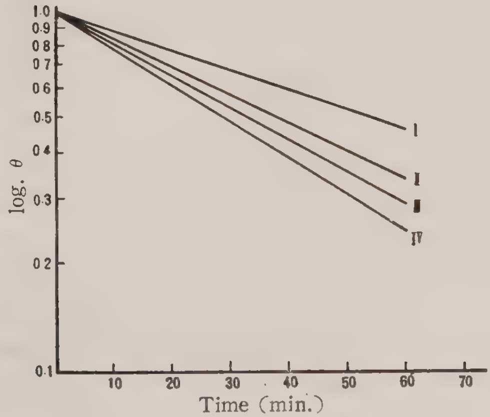


Fig. 5. The value of the coefficient of the cooling velocity of various canned foods

Note: I = canned crab, II = canned salmon, III = canned tuna, IV = canned sardine

Table 5. The values of the coefficient of the cooling velocity of various canned foods

Canned foods	The values of coefficient of the cooling velocity (n)
Canned crab	0.0146
Canned salmon	0.0191
Canned tuna	0.0229
Canned sardine	0.0248

$$\theta = e^{-nt} \quad \cdots\cdots\cdots (1)$$

$$\theta = T/T_0 \quad \cdots\cdots (2)$$

Where T_0 is the initial temperatre of the canned foods (°F) after heating process, T is the temperature in the canned foods (°F) after cooling process, t is the cooling time, n is the coefficient of the cooling velocity of the canned foods.

As seen in Fig. 5, the velocity of cooling of canned sardine is largest, followed in order by canned tuna, canned salmon and canned crab. The heat conductivity of canned crab is worst. It is seen that a long time is required to complete the cooling. From the view point of the difference of the cooling velocity, cooling by C-method may be denominated the slowest, while that by A-method is the most rapid. In the case of cooling by B-method, at the initial period of the process the cooling velocity is as good as by A-method, but in the final period it is like that by C-method.

Here, the time requiring to pass through "**Zone of the formation of large crystals**" described in the previous papers[4, 6] was calculated on cooling curves as shown in Table 6.

Table 6. The time requiring to pass through "Zone of the formation of the large crystals"

Kinds of canned foods	A-cooling	B-cooling	C-cooling
Canned crab	20.0 (min.)	38.0 (min.)	45.5 (min.)
Canned salmon	17.0	28.0	26.0
Canned tuna	18.5	25.0	26.0
Canned sardine	7.0	18.0	17.0

As seen in Table 6, in the cooling by A-method, the time during which the cooling curve passes through "**Zone of the formation of large crystals**" is the shortest. But, in the cooling by C-method, the time is the longest, so the crystals formed are considered to be large and to grow until they become large ones. These facts as actually observed in the canned crab and canned salmon will be described in the next article of this paper.

2. The formation of the crystals in the course of cooling canned crab by the various methods

1) Sample

The legs of crab caught off Nemuro, Hokkaido were removed from the carapace, and boiled with crust in sea water. The meat was taken from the crust, packed in cans, seamed, and then sterilized. After sterilization, the sample cans were cooled by A-method (rapid cooling), B-method (medium cooling) and C-method (slow cooling). The cooled cans were brought to the laboratory and opened after 50 days' storage at room temperature for observation of the states of the crystals formed.

2) Experimental method

Each part, juice, meat and parchment paper, in the canned crab was separated after opening the can. The size and number of the crystals formed in 0.5 *cc* of the juice were estimated on a Thoma's haemacytometer under the microscope. The state of juice of the canned crab was transparent or semitransparent and slight rough, so the crystals in the juice were easily identified by the method described above.

The viscosity of the juice, the amounts of volatile basic nitrogen, total nitrogen, soluble magnesium and phosphate in the juice were estimated by the method described later. On the other hand, the size of the crystals formed on the parchment paper was observed by a low power microscope.

The determination of the viscosity was made by Ostwald's viscosimeter, the determination of the amount of the soluble magnesium was done by the oxine method[1], and the estimation of the amount of volatile basic nitrogen or total nitrogen was carried out by the usual method.

3) Experimental results

Results obtained are shown in Table 7.

As seen in Table 7, the size of the crystals both those formed in the juice and on the parchment paper was small and microscopical, but the number was large in the rapidly cooled cans. The size of the crystals was somewhat larger and the number was somewhat less in the slowly cooled cans. In the case of the slowly cooled cans, few but larger crystals were formed on the parchment paper.

3. The formation of the crystals in the course of cooling of canned salmon by various methods

1) Sample

Some fresh salmon of which the mean body weight was 2,580 *g* and the mean body length was 59.5 *cm* was employed as the material for preparing the canned salmon. The material was packed in cans, seamed and sterilized as usual. After sterilization, some cans were rapidly cooled in water in which ice blocks were put, for 20 minutes;

Table 7. The state of the crystals formed in the course of cooling canned crab by various method

Sample cooling method	States of juice					Crystals in juice			Crystals on pearchment paper		
	Viscosity (η/η_0)	V. B. N. (mg%)	Tot. -N. (%)	Amount of soluble Mg. (mg%)	Amount of soluble PO_4 (mg%)	Size (μ)	Number (per cc)	Form	Top (μ)	Side wall (μ)	Bottom (μ)
Rapid cooling	2.3	38.2	1.34	3.0	59	20×25	8×10^4	E	none	none	38×6
Medium cooling	2.3	31.5	1.36	3.4	43	31×15	3×10^4	C	none	57×25	40×25
Slow cooling	2.1	20.0	1.21	6.3	—	72×12	4×10^4	C	300×100	300×100	250×100~2000×1000

Note: E = amorphous crystal C = columnar crystal

other cans were cooled most slowly at the temperature at 25 °C. The changes of the temperature in the cans are shown in Table 8.

Table 8. Change of temperature at the center of cans which were cooled by various cooling velocity

Cooling time	Rapid cooling	Slow cooling
0 (min.)	91.5°C	91.5°C
1	66.0	91.0
3	52.0	90.0
5	43.0	88.2
10	33.5	84.2
12	29.5	——
15	26.0	81.0
20	19.0	77.5
60	——	55.1

2) *Experimental method*

The macrocrystals formed in the canned salmon are generally smaller and fewer than those formed in the canned crab as shown in the previous paper[1].

So the size and the number of the crystals were estimated for visible macrocrystals contained in a can of canned salmon by the method shown in Fig. 6 from

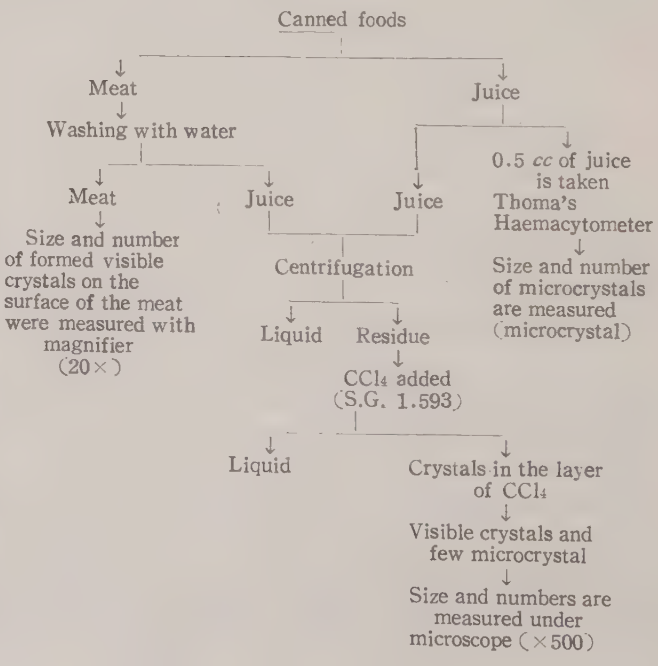


Table 9. The state of the crystals formed in the course of cooling canned salmon by various method

Cooling method	Sample No.	pH	Crystal in juice: Amorphous Size (μ)	Amorphous Number (/cc)	Microcrystal Size (μ)	Microcrystal Number (/can)	Visible crystal Size (μ)	Visible crystal Number (/cc)	Crystal on meat: Visible crystal Size (μ)	Visible crystal Number (/cc)
Very rapid cooling	1	6.2	16×16	many	25~65×8	5×10^4	450~1800×250~410	80	250~1800×32~410	85
	2	6.4	16~32×16	many	25~57×8~13	7×10^4	430~1550×250~490	60	450~1630×200~500	29
	3	6.4	49×16	many	—	none	450~5500×200~300	25	1000~5500×200~1500	7
Very slow cooling	1	6.2	16×16	few	50~110×13~25	3×10^4	450~5500×300×1000	56	500~6500×200~1800	77
	2	6.2	16×16	few	49~450×13×180	2×10^4	500~2460×170~570	25	500~2840×200~500	24
	3	6.1	32×16	few	49~100×12~16	7×10^4	2000~8000×1000~2000	8	—	none

the commercial point of view. For the observation of the state of the microscopic crystals in the juice, 0.5 *cc* of the juice was taken and the size and the shape of the microcrystals were estimated by Thoma's haemacytometer. Then the number of crystals was calculated to the number per 1 *cc* of the juice.

3) *Experimental results*

The observed state of the formation of the crystals during processes of cooling canned salmon by various methods is shown in Table 9.

As seen in Table 9, the size of the crystals either formed in the juice or on the meat was smaller, while the number was larger in the rapidly cooled cans than in the slowly cooled cans.

Discussion

As seen in the results obtained in this experiment either in the canned crab or in the canned salmon rapidly cooled after sterilization, the size of the formed crystals was smaller and the number was greater, but the size of the formed crystals was larger and the number was less in the slowly cooled cans.

These facts agree with the experimental results on the growing of the crystals in the test tubes and with the observed results on the growing of the crystals in the test tubes and with the observed results on the formation of the separaped crystals under microscope reported in the previous paper[6]. In the process of cooling after the sterilization in practical canning, it is evident that the amorphous crystals began to form at 70° ~ 50 °C due to the reaction among each

of the components (Mg, NH_4, PO_4) of the crystals. It is considered that crystals of the regular form are scarcely formed above 50°C, because at near 50°C the solubility of the crystal is maximum, and the stabilization of the supersaturated solution will result from the presence of high molecular substances (*e.g.* protein). This fact that the regular crystals are scarcely formed above 50 °C was practically seen during the process of cooling of canned crab by the microscopical observation of the crystals in the juice of the can at 60 °C taken onto the slide glass on which was a small hot water tank as shown in Fig. 1[6]. In the further progress of the reduction of temperature by cooling, when the temperature of the canned foods falls slowly in the range of 50° ~ 30°C which has been called "**Zone of the formation of large crystals**" in the previous paper[2], the larger and the more regular crystals are formed and further grow to the larger ones. On the contrary, when the canned foods are cooled rapidly passing through the 50° ~ 30°C range of temperature in a short time, the crystals will have no reserve to grow to become the large crystals, therefore many small crystals will be formed. The similar formation of the larger crystals is considered to occur during the storing of canned foods in the same range of temeprature. So, the storing temperature should be kept below that of "**Zone of the formation of the large crystals**", that is to say, should be kept below 20 °C.

III. Relation between the formation of the crystals and the freshness of the raw material

Whether the difference in the formation of the crystal may be caused by freshness of the raw material, or not has attracted much discussion. According to Matsuike *et al.*[7], no difference in the formation of the crystals was observed which could be attributed to the freshness. But, Nagai[8] said that the better the freshness of the raw material was, the more were the crystals formed in canned foods. Also, according to the recent report of Yamada and Fujii[9], the size of the crystals formed in canned crab which was made from unfresh raw meat was small and their number was few.

The authors have studied the relation between the formation of the crystals and the freshness of the raw material.

1. In the case of canned crab

1) Sample

Each sample of canned crab was prepared either from fresh material (volatile basic nitrogen was about 7 *mg%*) which was caught off Nemuro and was soon dealt with after landing, or from unfresh material which was left at room temperature for 2.5 days (volatile basic nitrogen was about 40 *mg%*) after landing. After usual sterilization, the cooling was done at rapid or slow velocities. The cans were brought to the laboratory and opened after keeping at 10° ~ 15°C for 50 days.

2) Experimental method

Experimental method was the same as that described in the previous article.

3) *Experimental results*

The obtained results are shown in Table 10.

As seen in Table 10, no remarkable difference in the state of the formation of the crystal was observed. However, the crystals formed in the juice of the cans prepared from unfresh raw material were larger than those from fresh raw material. On the contrary, the crystals formed on the parchment paper in the former ones were smaller than the crystals in the later ones.

Table 10. States of the formation of crystal in canned crab which were prepared from the raw material of various freshness

Cooling method	Freshness of material	States of juice						Crystals on perchment		
		Vis-cosity (η/η_0)	Soluble Mg (*mg%*)	Soluble PO_4 (*mg%*)	Crystal in juice					
					Size (μ)	Number (/*cc*)	Form	Top (μ)	Side wall (μ)	Bottom (μ)
Rapid cooling	Fresh	2.2	3.0	59	20×25	8×10^4	E C	none	none	38×6
	Unfresh	2.3	2.4	52	38×8	24×10^3	E C	none	11×4	16×8
Slow cooling	Fresh	2.2	3.4	42	31×15	31×10^4	C E	none	57×25	40×25
	Unfresh	2.0	2.5	43	38×9	25×10^5	C E	38×25	38×25	25×8

Note: E=amorphous crystal, C=columnar crystal

2. In the case of canned salmon

1) *Sample and method*

On a floating cannery, some canned salmon was prepared either from fresh material of half bodies which was promptly dealt with after catching, or from unfresh material of the other half bodies, which was left at the temperature of 17 °C for 28 hours after catching. After usual sterilization, the cans were slowly cooled with showering. The cans were brought to the laboratory and left at room temperature for 70 days and then opened. Experimental method was the same as that described in the previous article.

2) *Experimental results*

The obtained results are shown in Table 11. As seen in Table 11, the number of crystals formed in the cans which were prepared from unfresh material was somewhat less than that in the cans prepared from fresh material, but no remarkable difference in the size of crystals formed in the two types of material was observed.

Discussion

Using canned crab and canned salmon, the authors have observed the relation between the formation of the crystals and the freshness of the raw material. Some difference was observed between cans prepared from fresh and unfresh raw material. The size of

Table 11. State of the formation of crystal in canned salmon which were prepared from the raw material of various freshness

Freshness of raw material	Sample No.	pH	Crystal in juice: Amorphous Size (μ)	Amorphous Number	Microcrystal Size (μ)	Microcrystal Number	Visible crystal Size (μ)	Visible crystal Number	Crystal on meat: Visible crystal Size (μ)	Visible crystal Number
Fresh	1	6.0	16×16	few	48~110×13~25	3×10^4 /cc	450~5500×300~1000	56	500~6500×200~1800	77/can
Unfresh	1	6.2	16×16	few	32~64×16	3×10^4	500~6000×200×800	9	2000~5000×200~1000	10
Fresh	2	6.2	16×16	few	49~450×13~180	2×10^4	500~2460×170~570	28	500~2840×200~590	24
Unfresh	2	6.1	16×16	few	48~110×13	4×10^4	500~3000×200~1000	220	400~3000~×200~800	47
Fresh	3	6.1	16×16	few	32~160×16~96	7×10^4	1000~6000×1000~2000	8		none
Unfresh	3	6.2	16×16	few	48~110×130	2×10^4	1500×800			none
Fresh	4	6.1	16×16	few	32~62×16~32	7×10^4	1000~1500×500	15	2000~3000×1000	2
Unfresh	4	6.2	16×16	few	48×8	2×10^4	500~2000×500	6		none

the visible crystals formed in the canned crab prepared from unfresh meat was smaller than that from fresh raw material, but, no difference in the size of the formed crystals in the canned salmon was observed. Also in comparison with the cans prepared from fresh raw materials, numerous crystals were formed in the cans prepared from unfresh meat. However, as seen in Tables 9 and 10, the state of crystallization was considerably influenced by the velocity of cooling, in comparison with the influence of the freshness of the raw meat. In the cans cooled slowly, the formed crystals were larger in size and less in number, regardless of the freshness of the raw material.

If the conclusion is reached that crystals larger in size and fewer in number are obtained in cans packed with unfresh meat, it will be considered that this occurs owing to the difference of the amount of Mg in the fresh meat and in the unfresh raw meat. In the unfresh meat, the amount of Mg decreases owing to the decomposition and the fragillization of the tissue.

Accordingly, the authors have undertaken to determine the amounts of magnesium contained in canned crab and canned salmon which were prepared from the

raw materials of various degrees of freshness. In the canned crab prepared from fresh raw meat, total amount of magnesium was 0.037% and was 0.173% in the anhydrate. But, in the canned crab prepared from unfresh raw material, the amount of magnesium was 0.030% and was 0.151% in the anhydrate. In the canned salmon prepared from fresh or unfresh raw material, total amounts of the magnesium was 0.020% (0.058% in the anhydrate) in the former and 0.011% (0.030% in the anhydrate) in the latter. The obtained results are shown in Table 12.

Table 12. Total amount of magnesium in canned food prepared from the raw material of various freshness

Canned foods		Total amount of Mg (%)	Total amount of Mg in anhydrate (%)
Canned crab	Fresh	0.037	0.173
	Unfresh	0.030	0.151
Canned salmon	Fresh	0.020	0.058
	Unfresh	0.011	0.030

As seen in Table 12, a considerable difference of the total amounts of magnesium was observed between cans prepared from fresh and unfresh raw material. The amount of magnesium contained in the can prepared from fresh meat was more than in that from unfresh raw material. As the tissue of unfresh meat was fragile, the protein was denaturated as noted above. The reduction of the amount of magnesium in the cans prepared from unfresh raw material was considered to be owing to the flowing out during the washing process. Then, the conclusion is that the state of the formation of crystals in the cans prepared from the different freshness of the raw material is considerably influenced by the reduction of the magnesium.

IV. Relation between the formation of the crystals and the difference caused by use of fresh water or sea water for boiling during the processing of canned crab

In the previous article of this paper, the authors have observed that the amount of Mg indicated the largest value both in the crab meat and in the boiling water, in the case of the use of sea water for the processing of canned crab. In this paper the authors report observation on the difference of the formation of the crystal of $MgNH_4PO_4 \cdot 6H_2O$ by the employment of sea water or fresh water.

1) Experimental method

Canned crab was prepared by the usual method. In processing of the can, sea water and fresh water were used for boiling the meat. After the sterilization three cooling methods were used: rapid cooling (A-method), slow cooling (B-method) and very slow cooling (C-method), as described in the previous article. The canned crab thus prepared was brought to the laboratory and opened after 60 days storage. The conditions of the

Table 13. The state of formation of crystal in canned crab which are prepared by using sea water or fresh water for boiling

Boiling water		Fresh water			Sea water		
Cooling method		Rapid cooling	Medium cooling	Slow cooling	Rapid cooling	Medium cooling	Slow cooling
States of meat	Color	discolored	discolored	discolored	good	good	good
	Taste	watery	watery	watery	good	good	good
Viscosity		2.3	2.3	2.1	—	3.1	4.3
V. B. -N. (*mg%*)		38.2	31.5	10.05	31.9	30.2	26.1
Total-N (%)		1.34	1.36	1.46	1.29	1.10	1.21
Soluble Mg (*mg%*)		3.0	3.4	—	3.4	1.7	6.4
Soluble PO_4 (*mg%*)		60	43	—	46	45	—
Crystals in juice	Number / Size (μ)	80×10^4 (E) 8×10^4 (C) 11×1 (E) 20×25 (C)	100×10^4 (E) 80×10^4 (C) 16×12 (E) 31×15 (C)	1000×10^4 40×10^4 (C) 10×3 (E)	64×10^4 (E) 16×10^4 (C) 9×6 (E) 30×9 (C)	80×10^4 (E) 40×10^4 (C) 10×12 (E) 50×12 (C)	10×10^4 (C) 25×3 (C)
	Form	E C	C	C	E C	E C	C
Crystals of the parchment paper	Top	none	none	300×100	none	none	300×100
	Side wall	none	57×25	300×100	none	190×100	300×100
	Bottom	60×38	40×25	$250\sim2000\times100\sim1000$	80×50	200×130	2000×1000

crab meat (color, taste), the viscosity of the juice, the amount of volatile basic nitrogen, total nitrogen, Mg and PO_4 were estimated. The determination of the amounts of Mg and PO_4 was carried out by the method described in previous paper.[2]

2) *Experimental results*

Results obtained are shown in Table 13.

As seen in Table 13, in the case of the use of fresh water for boiling, the canned crab meat showed light red color as well as discolored, also watery taste lacking in good flavor. On the contrary, when sea water was used for boiling, the meat was beautiful red and had good flavor. In respect to the difference of the formation of the crystal, in the case of use of sea water for boiling, in spite of larger amount of Mg dissolved in the juice of the can, the size, shape and number of the crystals formed in the juice showed no difference from that in the use of fresh water. However remarkable differences of the formation of the crystal were observed with the differences of cooling velocity even in materials prepared with the same kind of water (sea water or fresh water). That is to say, whether in the using of sea water or fresh water for boiling, the more rapidly the cooling was completed, the smaller the size of the crystal was.

Considering from the results obtained, it is better to use sea water for the boiling for the sake of the color and taste, as no difference was seen in the size of the crystals. Rapid cooling after the heating process must be recommended because the size of the crystals keeps small.

Summary

When the various kinds of water and salt solutions were used for boiling, the amount of Mg dissolved was 0.0028%. There is almost no difference in this respect among the kinds of water and salt solutions. The loss amount of Mg in crab meat by boiling was about 26%. The amounts of Mg and PO_4 in crab meat and in the juice separated by boiling were maximum in sea water being twice the values obtained in the use of distilled water and $BaCl_2$ or $CaCl_2$ solution.

The velocity of the cooling of canned foods after heating process is influenced according to the various cooling methods or to the kinds of canned foods. Temperature of the cans falls rapidly in the cooling tank having a large amount of water but when the canned foods are cooled by air-blast by electric fun, the temperature of the cans falls slowly.

If the canned foods are slowly cooled taking a long time to pass through the range of the temperature $50^\circ \sim 30\,^\circ C$, which is called "**Zone of formation of the large crystal**", the formed crystals are comparatively larger and fewer. But, when the canned foods are cooled rapidly passing through the $50^\circ \sim 30\,^\circ C$ range in a short time, the formed crystals are comparatively smaller in size and more in number.

Thus, it is important to cool canned foods rapidly after the heating process.

In the cans prepared from raw materials of different degrees of freshness the difference between the state of the formation of the crystals was observed to be dependent only upon the difference of freshness of raw material. However, it was observed that the crystals formed in the cans prepared from unfresh raw material were smaller in size and more in number. But, after all the differences caused by degrees of freshness of raw material were less than the difference caused by the various velocities of cooling.

The authors have observed the difference of the formation of the crystals in canned crab which was prepared by using sea water or fresh water and by cooing rapidly or slowly after the heating process. According to the results obtained, when sea water was used for the boiling, the quality of the canned crab becomes better than when fresh water was used.

More remarkable difference of the formation of crystals was influenced by the velocity of the cooling than by the difference in use of the kind of water. Even if fresh water was used for the boiling, the minimum amount of Mg was present sufficient to form the crystal in the juice; therefore sea water should be used, and the product cooled rapidly to prevent growth of crystals to larger size.

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ON FISH TROPOMYOSIN

I. Isolation and Amino Acid Composition

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Tropomyosin, which is one of the proteins to form part of muscle fibrils, was first discovered by Bailey[1] in 1946. It has been crystallized from muscles of several animals by dehydrating the minced muscle with organic solvents; the detailed properties[2)-7)] have been published on rabbit muscle tropomyosin.

In fish, Hamoir[8] has isolated tropomyosin and nucleotropomyosin from carp muscle without preliminary dehydration and lately by means of electrophoresis has found two kinds of tropomyosins in salt extract.[9] In the invertebrates, it has been also crystallized from squid,[10] octopus and pinna.[11] The amino acid composition, sedimentation and electrophoretic behaviors of squid muscle tropomyosin were described by Yoshimura.[10]

Recently fish muscle proteins have been investigated electrophoretically from the view of point of comparative biochemistry.[9] As fish represent the lowest classes of vertebrates, it should be permissible to draw general conclusions on the muscle proteins of vertebrates by comparison with fish muscle proteins and those of other animals'. In this study tropomyosin which can be obtained in high degree of purity from muscle fibrils is crystallized from haddock muscle by Bailey's method[2] and its amino acid composition is reported.

EXPERIMENTAL

Isolation and purification

There have been found two kinds of methods to extract and isolate tropomyosin from muscle, Bailey's method[2] and Hamoir's.[8,12] The former method employs minced muscle for dehydration with organic solvents after which tropomyosin is extracted with high concentrated salt solution at neutral pH; and in the latter method, tropomyosin is extracted by the rather acid salt solution unfavarable for the extraction of myosin.

In the present experiment, tropomyosin is prepared from haddock muscle by Bailey's method.[2] The fresh haddock muscle, in which tropomyosin contents comes to 0.34 per cent of fresh weight and the nitrogen is 2.99 per cent of total protein nitrogen, was kept in frozen state about -15 °C. to -20 °C. for two months. This treatment denatures actomyosin as completely as, and the isolation of tropomyosin is more easy. Minced muscle is washed with water and dehydrated in ethanol and ether. The fiber is extracted with M-KCl at pH 7 to give viscous extract. Step (1). The protein is precipitated at pH 4.6. Step (2). The precipitates are redissolved at pH 7 and impurity is removed by adding ammonium sulphate to 41 per cent saturation. Step (3). The mother liquid is further saturated to 75 per cent saturation, and the protein is salted out from the mother

liquor. The dialysed tropomyosin is purified by several method: the repetition of Step (1)-(3), heat treatment at neutral pH, crystallization and the treatment of ethanol and ether. The purity of several preparations is shown as Table 1.

Table 1. The purity of several preparations

Method	Nitrogen content (%)	Tryptophan content* (%)
Once performance of Step (1)-(3) and once crystallization	16.9	0.13
Once performance of Step (1)-(3) and twice treatment of ethanol and ether	16.8	0.04
Once performance of Step (1)-(3) and heat treatment at 100 °C., pH 7	16.7	trace
Thrice performance of Step (1)-(3)	16.8	0.04
Thrice performance of Step (1)-(3) and twice treatment of ethanol and ether	16.7	0.02
Thrice performance of Step (1)-(3) and thrice crystallization	16.7	0.01

* Tryptophan is estimated by means of the use of p-dimethyl amino benzaldehyd.

As tryptophan is not included in fish tropomyosin as well as in rabbit's, the tryptophan content is an index of the purity of the protein. The salted out protein of the first carrying out of Step (1)-(3) is easily crystallized by dialysis against a salt solution containing 16 g. ammonium sulphate per 1 liter and 0.01 M acetate buffer, pH 5.4 at 0 °C. This preparation contains some impurities and crystals in large plate are obtained as shown in Fig. 1 (a). However, the continued repetition of the Step (1)-(3) and thrice crystallization results in good purity and the form of elongated prisms as shown in Table 1 and Fig. 1 (b). Nine grams of this crystal are obtained from 1 kg. minced muscle. The elementary analysis is given in Table 2.

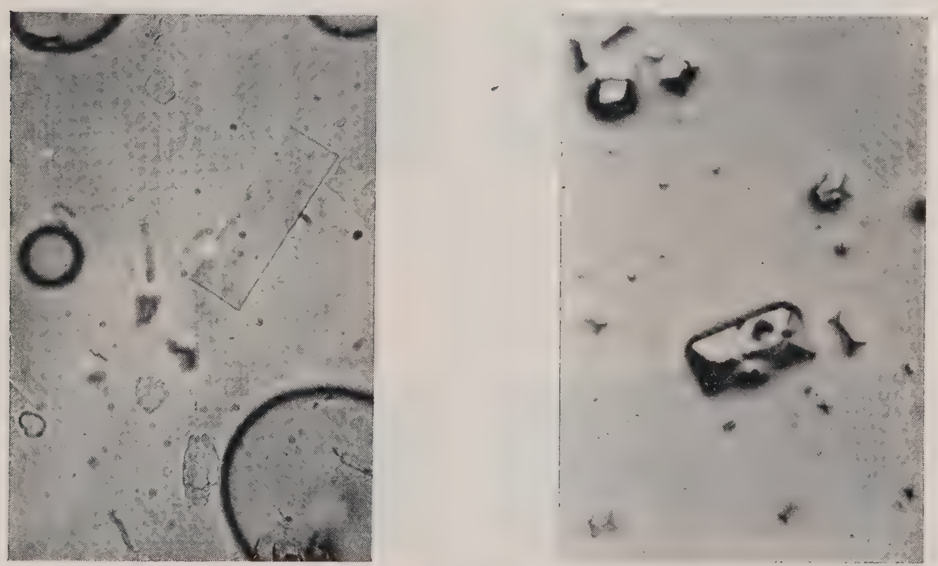
Electrophoretic analysis

The electrophoretic test is performed by Tiselius apparatus (cell size, 2 × 15 × 50 mm.) on the preparations as described above.

The obtained results are shown as Table 3 and Fig. 2. The preparation obtained from once carrying through of Step (1)-(3) and crystallization includes more impurity than that of thrice repeated Step (1)-(3) like the results obtained from the test of tryptophan contents. This impurity migrates with a mobility of about 70 per cent of that of tropomyosin. The preparation derived from thrice repetition of Step (1)-(3) and thrice crystallization has a high purity and a single boundary in glycine-HCl buffer and phosphate buffer, I=0.15, I=0.35, pH 2.5-3.5 and pH 5.9-8.0. The pH-mobility curve found is graphed in Fig. 3; the isoelectric point lies near pH 5.1 from the extrapolation.

(a) Crystals of once performance Step (1)-(3) and crystallization

(b) Crystals of thrice performance Step (1)-(3) and thrice crystallization



(× 180)

Fig. 1. Tropomyosin crystals from haddock skeletal muscle

Table 2. Elementary analysis of haddock tropomyosin

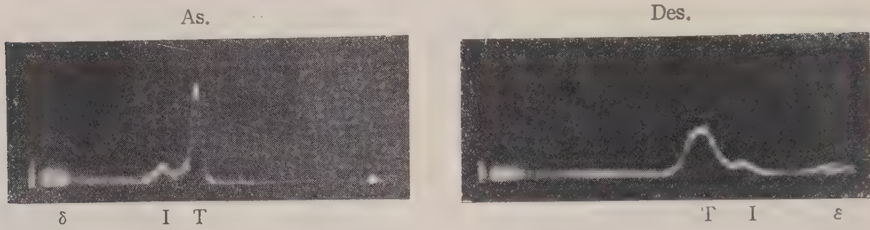
Nitrogen (%)	Sulphur (%)	Phosphorus (%)
16.7	0.76	0.03

Table 3. Electrophoretic analyses of preparations

Preparation		Tropomyosin		Impurity	
		ratio (%)	mobility*	ratio (%)	mobility*
Once performance of Step (1)-(3) and crystallization	Descending	83	-6.50	17	-4.44
	Ascending	83		17	
Thrice performance of Step (1)-(3)	Descending	95	-6.53	5	-4.39
	Ascending	92		8	

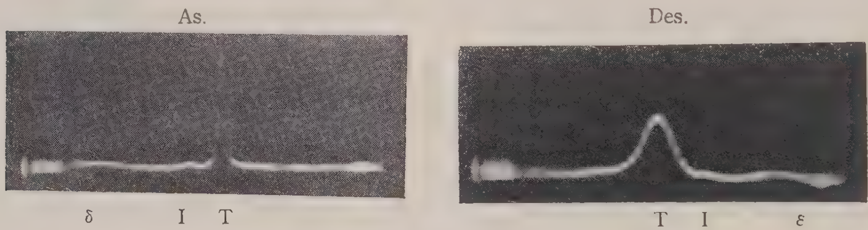
* $\times 10^5$ cm^2/sec. volt.

(a) The preparation of once performance Step (1)–(3) and crystallization



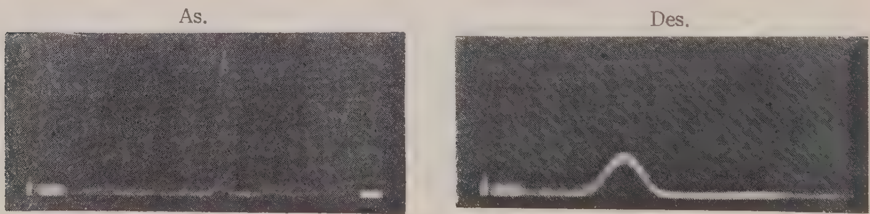
I=0.15 (phosphate, 0.1 and NaCl, 0.05) protein conc., 1.25% pH 7.40
3.98 volt/cm. 7200 sec.

(b) The preparation of thrice performance Step (1)–(3)

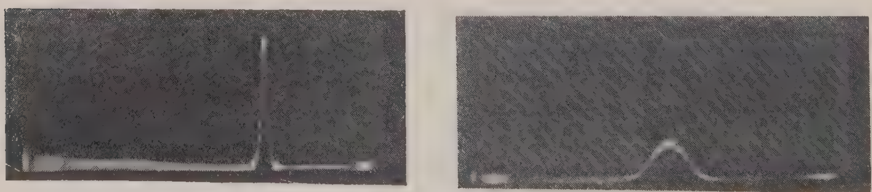


I=0.15 protein conc., 1.25% pH 7.40 3.98 volt/cm. 6308 sec.

(c) The preparation of thrice performance Step (1)–(3) and thrice crystallization



I=0.15 protein conc., 0.6% pH 7.70 4.30 volt/cm. 7230 sec.



I=0.15 (glycine-HCl, 0.1 and NaCl, 0.05) protein conc., 0.06% pH 2.91
4.44 volt/cm. 7200 sec.

Fig. 2 Electrophoretic diagram of haddock tropomyosion
(T : Tropomyosin I : Impurity)

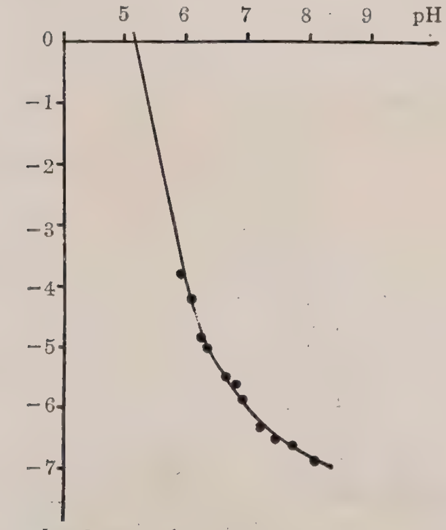


Fig. 3 pH-Mobility curve of haddock tropomyosin
〔I=0.15 (phosphate, 0.1 and NaCl, 0.05), and calculated from descending boundary〕

Amino acid composition

In the amino acid analysis of protein, the values of serine and threonine have been corrected by 10 per cent and 5 per cent for decomposition during hydrolysis according to the report of Rees.[13] The destruction rate of these amino acids is not only due to kinds of proteins, but the other amino acids are also decomposed by hydrolysis in 6 N HCl. The use of two periods of hydrolysis has been proved desirable.[14)–17)]

The tropomyosin, which is purified by the thrice repetition of Step (1)-(3) and crystallization, is dialysed against water and dried in freezing. After disolution of the protein in pyrex tube in 200 volumes of re-distilled 6 N HCl, the solution is frozen in a bath of solid carbon dioxide and ethanol: the tube is evacuated to 10 mm. pressure and sealed. Hydrolysis is performed in an oven at 110 ± 2 °C. for 22 and 72 hours. The slight yellow hydrolysate is filtered on glass filter. Excess HCl is removed by repeated drying in vacuo; the residue is washed into volumetric flask and made up to desired volumn.

The chromatography of the hydrolysates is performed on Dowex 50 (X-8) column by the procedure of Moore and Stein,[18] except for no addition of detergent to all buffers and the elution of tyrosine and phenylalanine at 50°C. instead of 75°C. to avoid cracking the column. A 0.9 × 100 cm. column is used for acidic and neutral amino acids and 0.9 × 15 cm. column for the basic amino acids and amide nitrogen. In preliminary experiment, each amino acid in synthetic mixture is sufficiently eluted. Cystine and cysteine are determined on Dowex 2 column, 0.9 × 15 cm., as cysteic acid after oxidation of the protein by performic acid.[19] Micro Kjeldahl analysis on the hydrolysates give the nitrogen value on which percentile values are based. To convert of terms of protein weight, the nitrogen value of 16.7 per cent is used for tropomyosin. Amide nitrogen is determined independently in order to check the values calculated from the chromatographic results.

The elution curves of long column of 22 and 72 hours hydrolysates are given in Figs. 4 and 5. In the case of 22 hours hydrolysate, the variations in the base line of the elution curves are remarkably in comparison with 72 hours', especially before and after the valine: unknown asymmetric peaks obtained are shown in Table 4.

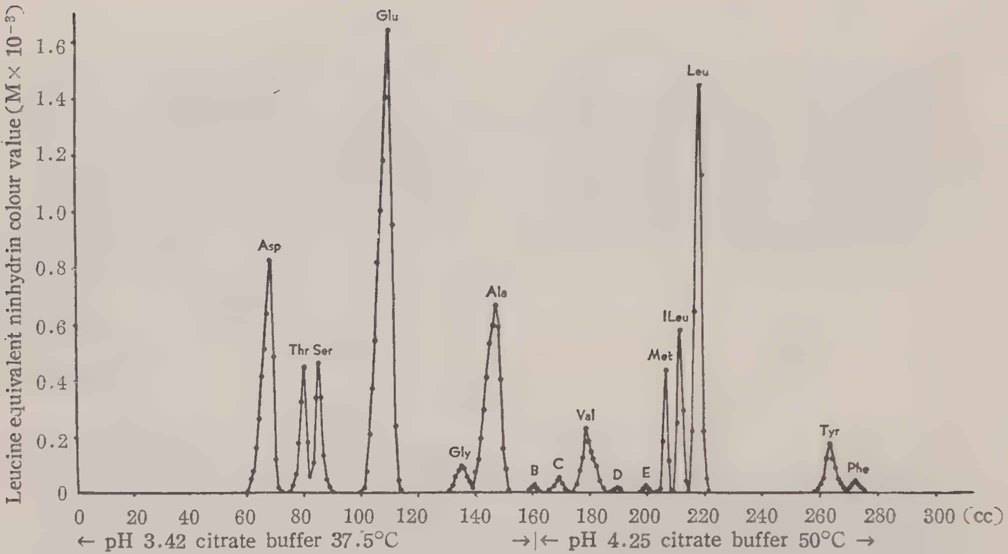


Fig. 4 Elution curve for a 22 hours hydrolysate of tropomyosin on a 0.9×100 cm. Dowex-50 (x-8) column (4.24 mg. of protein hydrolysate is used)

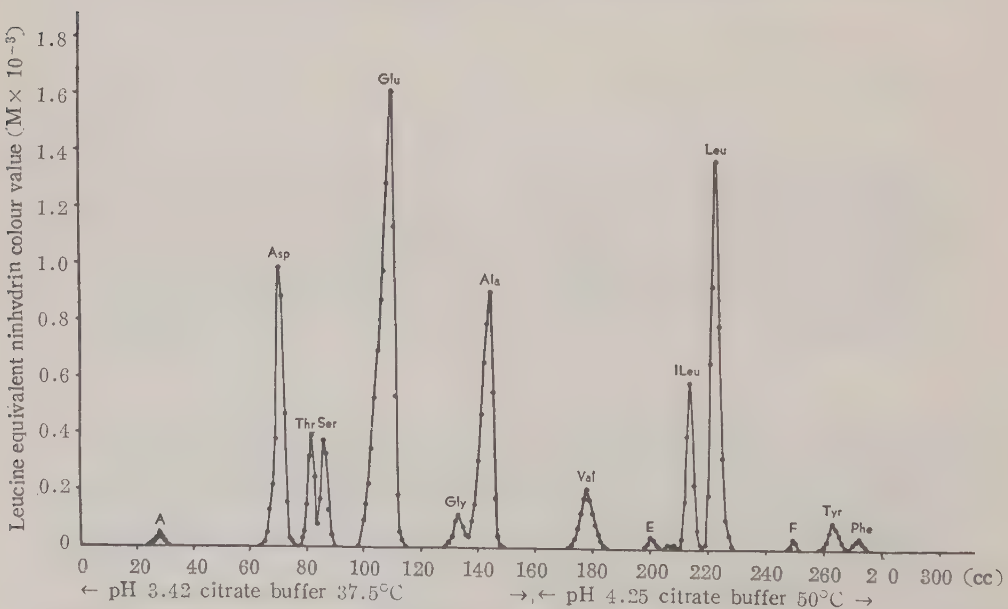


Fig. 5 Elution curve for a 72 hours hydrolysate of tropomyosin on a 0.9×100 cm. Dowex-50 (x-8) column (4.23 mg. of protein hydrolysate is used)

All peaks in Table 4 are not identified, but C and D, which are eluted before and after the valine in 22 hours hydrolysate and disappeared in 72 hours', should be certain peptides in view of the facts that compounds in which the carboxyl group of isoleucine

Table 4. Unknown peaks in hydrolysates

Fraction no. (cc.)	22 hours hydrolysate (N%)*	72 hours hydrolysate (N%)*
25 (A)		0.24
160 (B)	0.17	
170 (C)	0.38	
190 (D)	0.10	
200 (E)	0.20	0.22
250 (F)		0.15

* Nitrogen as per cent of total protein nitrogen in hydrolysate and the average value of three analyses

or valine are coupled in peptide linkage are not easily hydrolyzed by HCl and these peptides are eluted near the valine peak, On the other hand, peak E shows no variation in amounts of nitrogen per cent. As the change of buffer is performed at fraction number 160 cc. and the replacing of the buffer in column is completed at 200 cc., it seems to be an artifact which is eluted out.

On the short column, the variation of the base line after ammonia is both extreme in 22 and 72 hours hydrolysates as shown in Figs. 6 and 7.

In Table 5 are presented the analytical results calculated from three pairs of samples of 22 and 72 hours hydrolysates of tropomyosin on Dowex 50 columns. A comparison of the analytical values of each hydrolysate indicates certain decomposition of serine, threonine, tyrosine and lysine as the hydrolysis time is lengthened. Fxpecially, methionine is almost decomposed by hydrolysis and irregular peaks appears at the original position of methionine. On the other hand, amounts of valine, leucine, isoleucine and ammonia are increased by prolonged hydrolysis.

Assuming that the loses of amino acids accompany with the first order kinetic, the extrapolation to zero-time is used for the values of serine, threonine, tyrosine and lysine. But no calculation is performed for glutamic acid and ammonia because of their large average deviation. For the value of ammonia is applied that of independent analysis. For aspartic acid, glutamic acid, glycine, alanine, phenylalanine, histidine and arginine are adopted the average values of 22 and 72 hours hydrolysis, and the resuts of 72 hours for the values of valine, isoleucine and leucine. Then, for the value of methionine is applied the result of 22 hours' hydrolysis. The amino acid composition of tropomyosin is summarized in Table 6 from these results.

DISCUSSION

Haddock tropomyosin is easily crystallized in the form of a long prism from the salted out precipitates, depending upon the presence of salts to break down the large

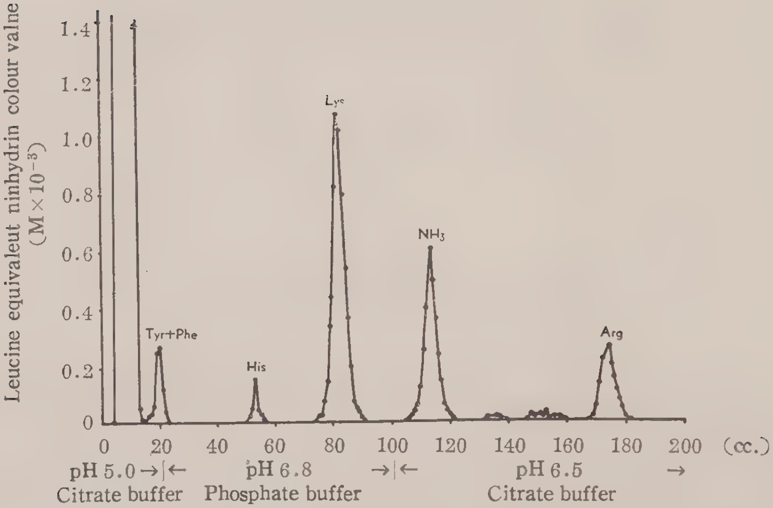


Fig. 6. Elution curve for a 22 hours hydrolysate of tropomyosin on a 0.9×15 cm. Dowex-50 (x-8) column (4.24 mg. of protein hydrolysate is used)

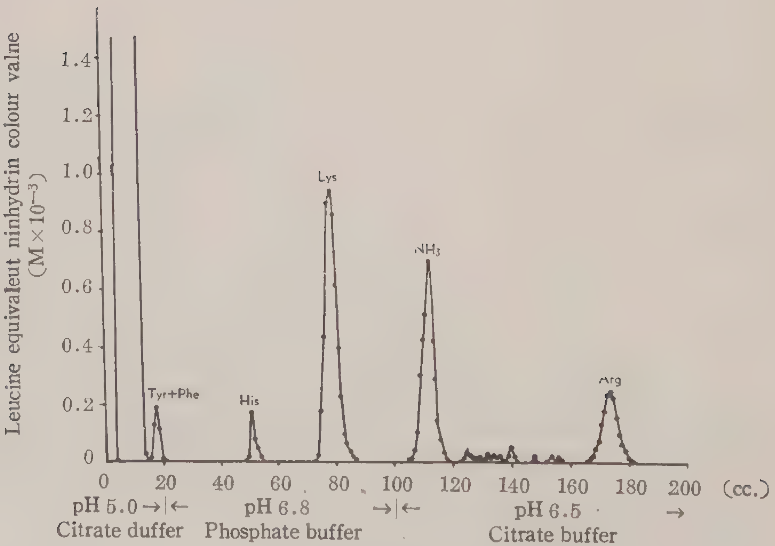


Fig. 7. Elution curve for a 72 hours hydrolysate of tropomyosin on a 0.9×15 cm. Dowex-50 (x-8) column (4.23 mg. of protein hydrolysate is used)

Table 5. Effect of hydrolysis time on amino acid composition of tropomyosin

Amino acid	Nitrogen as per cent of total protein nitrogen	
	22 hours hydrolysate	72 hours hydrolysate
Asp	7.16±0.17	7.14±0.11
Thr	2.69±0.02	2.28±0.02
Ser	2.96±0.04	2.24±0.04
Glu	20.04±0.30	19.63±0.40
Gly	0.99±0.05	1.01±0.03
Ala	8.55±0.01	8.38±0.05
Val	2.06±0.10	2.44±0.04
Met	1.48±0.06	
Ileu	2.31±0.04	2.69±0.04
Leu	7.48±0.01	8.45±0.00
Tyr	1.36±0.03	0.74±0.02
Phe	0.30±0.01	0.28±0.02
His	1.57±0.09	1.64±0.08
Lys	17.68±0.02	16.65±0.05
Amide-N	5.57±0.33	6.10±0.32
Arg	12.54±0.11	12.67±0.15

Analytical values are obtained from 4.23–4.24 mg. of protein hydrolysate and from 5.01 mg. protein hydrolysate for Gly., Tyr. and Phe.

Table 6. Amino acid composition of tropomycsin

Amino acid	Amino acid per 100g. of protein	Amino acid residue per 100 g. of protein	N as per cent of protein N	Moles of amino acid per 10^5 g. of protein	
Asp	11.36	9.82	7.15	85.4	85
Thr	4.11	3.49	2.89	34.4	34
Ser	4.19	3.47	3.34	39.8	40
Glu	34.82	30.57	19.84	236.6	237
Pro	0	0	0	0	0
Gly	0.90	0.68	1.00	11.9	12
Ala	9.00	7.18	8.47	101.0	101
Val	3.41	2.89	2.44	29.1	29
Met	2.64	2.32	1.48	17.6	18
Ileu	4.22	3.64	2.69	32.1	32
Leu	13.26	11.44	8.45	101.0	101
Tyr	3.88	3.49	1.79	21.4	21
Phe	0.58	0.52	0.29	3.5	3.5
His	0.99	0.88	1.61	6.3	6
Try	0	0	0	0	0
Lys	16.64	14.59	19.05	113.8	114
Amide-N	0.86*		5.15*	61.0*	61*
Arg	6.57	5.89	12.61	37.7	38
Cys/2	0.78	0.65	0.55	6.5	6.5
Total	117.35	101.52	98.80		878

* Omit from the total value

aggregates. The tropomyosin has a unique property toward salts: it can be depolymerized and fragmented by salt concentration and the crystals have to make enormus hydration just as in the case of rabbit tropomyosin. Because of this, the form of crystals and the purity are extremely effected by impurities; it is necessary to crystallize tropomyosin from precipitates which have been purified as completly as possible.

In Table 7, the electrophoretic mobility of haddock tropomyosin is compared with that of other origins. The mobility of haddock tropomyosin is rather in agreement with that of carp and is closer to rabbit's to squid's. These facts should be expected from the amino acid composition.

The amino acid composition of haddock, squid and rabbit tropomyosin is shown in Tables 8 and 9. Like rabbit and squid protein, haddock tropomyosin contains no tryptophan and proline. In the tropomyosins of invertebrates,[10,11] aspartic acid and the amide nitrogen is much higher and arginine appers to have replaced part of lysine, but these facts do not hold in the

Table 7. Electrophoretic mobilities* of tropomyosins

Buffer	Haddock	Carp[8]	Squid[10]	Rabbit[20]
I=0.35 (phosphate 0.1, NaCl 0.25) pH 7.1	-4.09	-3.90	-2.80	-4.9
I=0.15 (phosphate 0.1, NaCl 0.05) pH 7.4	-6.58	-6.20	-5.71	

* $\times 10^5 cm^2/sec.\ volt.$: calculated from descending boundary

Table 8. Comparative amino acid composition of tropomyosins

Amino acid	Moles of amino acid per 10^5g. of protein		
	Haddock	Squid*	Rabbit[7]
Asp	85	100	89
Thr	34	32.5	28
Ser	40	55	40
Glu	237	212	211
Pro	0	0	0
Gly	12	28	12.5
Ala	101	95	110
Val	29	25	38
Met	18	17	16
Ileu	32	19.5	29
Leu	101	95	95
Tyr	21	8	15
Phe	3.5	8	3.5
His	6	12	5.5
Try	0	0	0
Lys	114	90	110
Amide-N	(61)	(94)	(64)
Arg	38	68	42
Cys/2	6.5	6	6.5
Total	878	871	851

* Being corrected on data of Yoshimura[10] by the private communication of Dr. K. Bailey.
( ) Omit from the total value.

case of haddock tropomyosin. Hoddock tropomyosin is also more close to rabbit tropo myosin in amino acid composition than to squid, although some differences are recognized with respect the number moles of amino acid.

SUMMARY

Haddock tropomyosin is crystallized in high purity. The electrophoretic mobility is more close to that of mammalian tropomyosin than to invertebrate's, and simular facts are also recognized in the amino acid composition.

The author wishes to express his hearties thanks to Professor Katsuzi Yoshimura, Faculty of Fisheries, Hokkaido Univeraity, and to Professor Shiro Akabori, Faculty of Science, Osaka University, for their valuable advices.

Table 9. Amino acid group per cent of tropomyosins

Group	Haddock		Squid[10]		Rabbit*	
Free acid group	29.7	47.7	25.0	44.6	27.8	46.3
Base group	18.0		19.6		18.5	
Hydroxy group	10.8		10.9		9.8	
Amide group	7.0		10.8		7.4	
Polar group	66.2		66.9		64.3	
Non-polar group	33.8		33.1		35.7	

* Calculated from data of Kominz et al.[7]

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サメ肉腐敗の微生物化学的研究

第3報　尿素分解菌の尿素分解能力に及ぼす水素イオン濃度の影響について

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Studies in the Bacteriological Chemistry of Shark Muscle Spoilage

III. On the effects of pH upon the abilities of the ureasplitting bacteria isolated from spoiling shark muscle

Takahisa KIMURA

Abstract

In the present work, the author has observed the effect of pH upon the ability to decompose urea possessed by K-10 strain, which has especially stronger urease activity than the other four isolated urea-splitting bacteria.

The results can be summarized as follows;

1) The multiplication of this strain has been excellent at the range from 6 to 8 of pH values, and optimum growth pH at pH 7.9.

2) In this strain, optimum pH of urease activity lay within range of pH 7.0 to pH 8.2, and it changed slightly to the alkali side in accompaniment with increment of substrate concentration in reaction mixture.

This phenomenon was remarkable below 2% of substrate concentration, and in the case of 6% of substrate concentration, optimum pH lay pH 7.6, and in 0.6% at pH 7.6.

3) In this strain, the optimum pH in respect to ability of decomposition of urea will lay near pH 7.9, from the results obtained as above.

前報[1]に於て，著者は腐敗進行過程中に於ける鮫肉より尿素分解に比較的関係が深いと思われた細菌23株を分離し，その中5株が特異的にurease活性が強く，これ等の細菌は鮫肉に於ける尿素分解に主要な役割をなすであろうと報告した。本報に於てはその5株の中，就中活性の強いと思われたK-10株の尿素分解能力に及ぼす水素イオン濃度の影響について，2，3の実験を試みた。こゝでurease活性と云わず特に尿素分解能力としたのは菌体urease活性そのものが尿素分解能力を左右することは勿論であるが，一方その菌の発育の良否もその能力に大なる影響を及ぼすものと考えられるからである。細菌のurease活性に及ぼす水素イオン濃度の影響に関し内野等[2]は *Bac. subtilis* 等8菌種の乾燥菌体浮遊液の urease 活性と水素イオン濃度との関係について報告してその至適水素イオン濃度は *Bac. proteus* では pH 6～7，*Bac. coli* 及び *Bac. typhi* はpH 6～8，*Bac. pyocyaneus* 及び *Bac. prodigiosus* は pH 7～8，*Bac. subtilis, Staph. aureus* 及び *Bac. dysentheriae* は pH 8 であると述べて居る。又 Lyubimov[3]は *Microc. ureae* に於ける urease の至適水素イオン濃度は pH 7 より幾分低いところにあると述べて居る。いずれにしろ菌種によりその至適水素イオン濃度が異る事は明らかである。又 Sumner 等[1]は刄豆結晶 urease の至適水素イオン濃度は基質尿素の濃度により変化すると報告している。本報に於てはこの点についても検討を行つた。尚本実験に供試した K-10 株の一

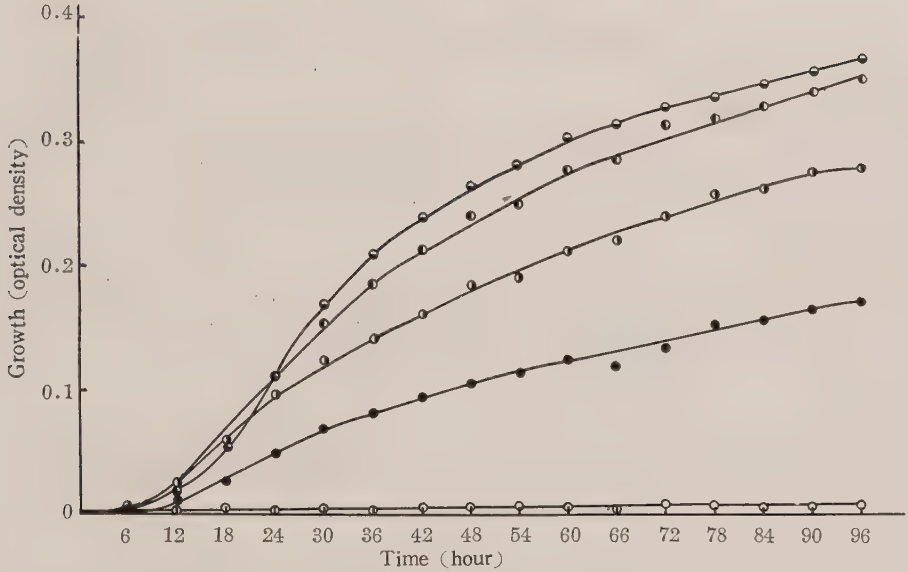


Fig. 1 The effect of hydrogen ion concentration on the growth rate of K-10 strain, at pH 5.8~7.8 with 0.067M phosphate buffer solution

○ pH 5.8 ● pH 6.4 ◑ pH 6.8 ◐ pH 7.5 ◒ pH 7.8

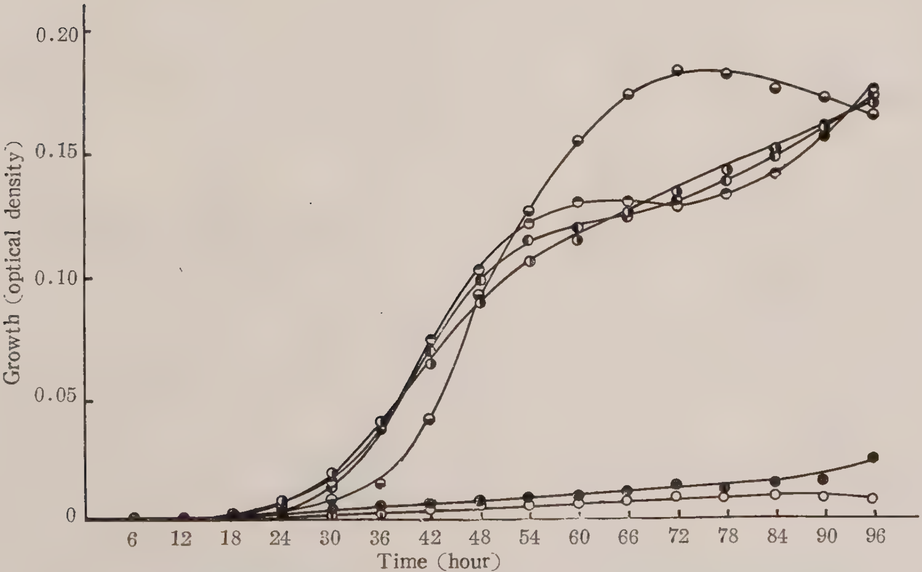


Fig. 2 The effect of hydrogen ion concentration on the growth rate of K-10 strain, at pH 6.8~8.8 with 0.067M KH_2PO_4+0.034M $Na_2B_4O_7$ buffer solution

◑ pH 6.8 ◓ pH 7.2 ◐ pH 7.5 ◒ pH 7.9 ● pH 8.4 ○ pH 8.8

般性質は古くから強力な urease 活性を有するとされて居る *Bac. proteus*, *Bac. ureae* 或は *Pseudomonas ureae* とは明らかに異つた桿菌であり，本供試菌株の分類学上の位置については後報に於て報告する予定である。

実験 I　K-10株の発育に及ぼす培地水素イオン濃度の影響

a) 使用培地

普通濃度の倍の濃度に調製した3% pepton 水に，pH 5.8〜7.8では終末0.067Mの燐酸ソーダ・燐酸カリ緩衝液，pH 6.8〜8.8では終末0.067M燐酸カリ，0.034M硼砂緩衝液を等量混合し，15ポンド20分間の加熱による少量の沈澱を濾別し，之を光学的に均一な比色用試験管に10cc宛分注して使用した。

b) 発育速度の測定法

上記培地に培養した供試菌の48時間培養液を適宜稀釈し，その0.1cc宛を上記培地に接種し，35°C の恒温器中で発育せしめ，6時間毎にその溷濁状態をA.K.A.光電比色計で750mμフィルターを使用して吸光度を測定する事により直接測定した。尚予備実験によればこの波長では培地自体による光吸収はほとんどみとめられず，溷濁度と吸光度は直線的関係にあつた。

c) 実験結果

以上の如き方法によりpH5.8〜7.8及び6.8〜8.8に於けるK-10株の発育を比較測定した結果は，Fig. 1及びFig. 2に示す通りである。即ち0.067M燐酸カリ・燐酸ソーダ緩衝液を用いたpH 5.8〜7.8の実験ではFig. 1に示す如くいずれのpHに於ても約6時間のlag phaseの後に発育を始め，培地のpHがアルカリ性に傾くにつれてその発育が旺盛となり，pH 7.8で最も旺盛な発育を示し，pH 5.8に於てはほとんどこの測定操作では測定し得る程度の発育を示さなかつた。同様に0.067M燐酸カリ，0.034M硼砂緩衝液で行つたpH 6.8〜8.8の実験ではFig. 2に示す如くいずれのpHに於ても約24時間のlag phaseの後に発育を始め，Fig. 1の場合と同様に，pH 7.9に於ては他のpHより幾分lag phaseが長いが，最も急速な発育を示し更にアルカリ度が高まりpH 8.4となると酸性側同様にほとんど発育が見受けられなかつた。即ち本菌種は約6〜8のpH領域で発育良好にして，特にpH 7.9附近に於て最も旺盛なる発育が認められた。

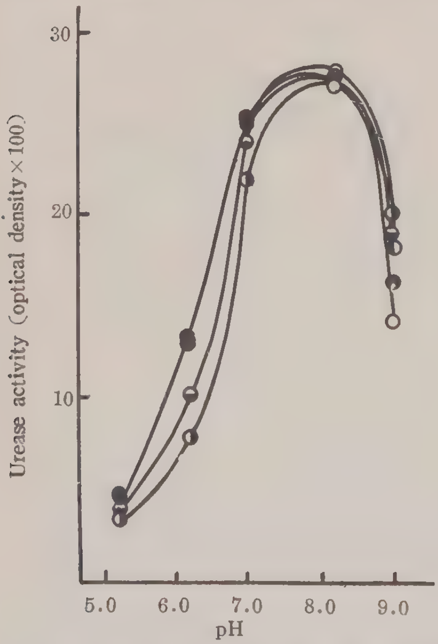


Fig. 3 The effect of hydrogen ion concentration and substrate concentration on the urease activity of intact cell suspension of K-10 strain, pH 5.2-9.0, substrate concentration 2%-10%
○ substrate 10%　◐ substrate 6%
◔ 〃 2%　● 〃 8%
◒ 〃 4%

実験 II　K-10株の菌浮遊液 urease 活性に及ぼす水素イオン濃度の影響及び基質濃度との関係

a) 酵素液の調製

前報[1]の結果によれば，本菌株の発育の際に於ける培地中の尿素の存否は菌体urease活性にほとんど影響をあたえなかつたので，本実験に於ては常法の如く普通肉汁寒天斜面培地に於ける30°C約24時間の培養菌を集菌し洗滌後調製せる冷水浮游液を酵素液として供試した。予備実験に於て，菌体浮游液及び凍結乾燥菌体を夫々+5°C以下の冷暗所及び室温に放置せる場合に浮游液の場合では急速に不活性化するに反し乾燥菌体の場合は冷暗所に保存すれば約5ヶ月後に於ても最初の½程度の活性を示し，相当の安定度を有することが判つたので本実験に於ては発育の際の条件差による影響を除くためにあらかじめ凍結乾燥し +5°C以下に保存した菌体を用に臨み5 mg/ccの濃度に冷水に浮

游せしめて供試した。

b）酵素活性の測定法

上記酵素液を用い Ambrose[5] の方法を若干改良した Nessler 比色法により測定した。即ち各濃度の基質尿素液 0.5cc と終末 M/8 各の pH 値を有する燐酸緩衝液 1 cc宛を試験管にとり 30°C の恒温に保ち10分後に酵素液0.5cc宛を添加し20分間反応せしめた後更に N/1 HCl 1cc宛を添加して反応をとめ，濾過した後濾液 1 ccに水18ccを加え之に Nessler 試薬を 1 cc 宛添加して10分後に 430mμ フィルターを用い A. K. A 光電比色計により吸光度を測定しこの吸光度から 0 時の吸光度を差引いたものを酵素活性度として表わした。

c）実験結果

以上の如き方法により菌体浮游液の urease 活性と水素イオン濃度及び基質濃度の影響を観察した結果は，Fig. 3，Fig. 4 及び Fig. 5 に示す通りである。即ち基質濃度が比較的高い 2 %から10%で，pH 5.2から9.0の場合での活性は Fig. 5 に示す如くで最適水素イオン濃度はいずれの基質濃度の場合も pH 7～8 にあるようであるが，基質濃度がうすくなるにしたがい pH 8 の方に幾分移動を示した。次に基質濃度を 2 %以下とした場合の活性は Fig. 4 に示す如くで明らかに基質濃度が減少するにしたがつて最適水素イオン濃度は pH

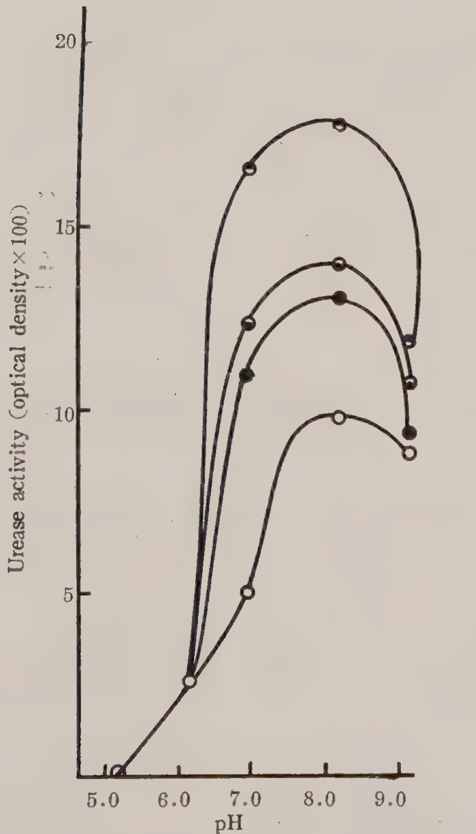


Fig. 4 The effect of hydrogen ion concentration and substrate concentration on the urease activity of intact cell suspension of K-10 strain, pH5.2-9.0. substrate concentration 0.6%-2.0%
○ substrate 0.6%　◒ substrate 1.6%
● 〃 1.0%　◓ 〃 2.0%

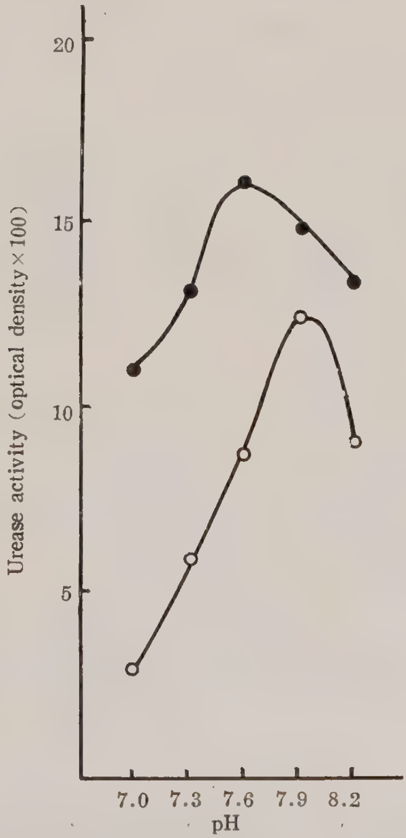


Fig 5 The variation in optimum hydrogen ion concentration of K-10 strain's intact cell suspension caused by substrate concentration
● substrate 6.0%
○ 〃 0.6%

8.2の側に移動しpH7.0での活性とpH8.2での活性の差は基質濃度の減少と平行して大きくなつた。又pH7.0からpH8.2の間に於ける活性度を見るためにその間を更に細分して基質濃度6%及び0.6%の際の至適水素濃度をよりこまかく検討した結果は，Fig. 5に示す通りである。即ち，基質濃度が高い時はその活性はpH7.6附近で，又低濃度の場合はpH7.9で最大となり，且ついずれの結果から見ても基質濃度が減少するにつれて活性も低下を示した。又Fig. 3及びFig. 4に見る如くpH 5以下の酸性域に於ては極端に活性は減少し，ほとんど之が認められなくなつた。

考　察

実験Iの結果によればK-10株の発育至適水素イオン濃度はpH7.9附近にあり，pH5.8以下の酸性領域又はpH8.4以上のアルカリ領域に於ては発育が阻害され又実験IIの結果によればurease活性の至適水素イオン濃度は，Sumner等[4]が提唱する如く基質濃度の影響を受けるがpH 7～8にあり，基質濃度終末2%以下に於てその影響は特に著しかつた。即ち基質濃度を低くするにつれ至適水素イオン濃度は幾分アルカリ性側に移動した。又酸性側に於てその影響は大であつた。又基質濃度が終末6%ではpH7.6前後，0.6%ではpH7.9前後に於てそれぞれ最大の活性を示し，須山等[6,7,8]が報告している鮫肉の尿素含量2%もしくはそれ以下に於てはpH7.6からpH7.9の前後で最も活性が大である。以上の結果から考察するに本菌株の尿素分解能力はpH7.0からpH8.2の間に於て最も大きく，特に鮫肉中の尿素濃度（2%前後）の点から考えて至適水素イオン濃度はpH7.9附近にあるのではなかろうかと思考される。又pH 5程度の酸性になると尿素分解能力は著しく阻害を受けるのであるが，大石[9]の研究からも明らかな如く極めて新鮮な鮫肉に於ては其の水素イオン濃度はpH5.6位の酸性域にあり，鮮度の低下にともない急速にアルカリ性に移動するので，先に著者の提唱した如く若しこのK-10株の如き細菌が鮫肉に於ける尿素の分解に主要な役割をなしているとすれば可及的新鮮な時期に於て，換言すれば鮫肉の水素イオン濃度がpH 5附近にある時期に何らかの手段を用いて，この種の細菌の発育を阻止するならば相当効果的に尿素分解を阻止し得ることが考えられ，又逆に鮮度低下による鮫肉pHのアルカリ性への移動はこれ等細菌による尿素の分解に対し好条件を与える結果となるものと考えられる。

要　約

前報[1]に於いて報告した鮫肉より分離せる尿素分解菌中，就中urease活性の強いと思われるK-10株の尿素分解能力に及ぼす水素イオン濃度の影響について観察し次の如き結果を得た。

1） K-10株はpH6からpH8の間に於て発育が旺盛で，発育の至適水素イオン濃度はpH7.9前後にある。

2） K-10株のurease活性の至適水素イオン濃度はpH7.0からpH8.2の間にあり，反応時の基質濃度が大となるにつれアルカリ性側に幾分移動する。この現象は基質濃度終末2%以下で著しく6%ではpH7.6，0.6%ではpH7.9附近で最も活性が大である。

3） 1)，2)の結果からK-10株の尿素分解能力の至適水素イオン濃度はpH7.9前後にあるものと考えられる。

終りに臨み，本報告の御校閲及び御指導を賜つた本学谷川教授並びに坂井助教授に深甚の謝意を表する。
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CHANGES IN TISSUE CHLORIDE AND PHYSIOLOGICAL ACTIVITY OF THE BRACKISH-WATER BIVALVE, *CORBICULA JAPONICA*, IN RESPONSE TO VARIATIONS IN SALINITY

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I. Introduction

The brackish-water bivalve, *Corbicula japonica*, lives in shallow estuarine waters, inlets and brackish lakes. In general, the adult clam is buried on the bottm surface with both their siphons protruding. However, in winter, this animal is found in bottom materials at depths ranging from 4 to 10 cm layer.

In Zyusan-gata Inlet, as already reported in a previous paper (Fuji, 1955), the chlorinity of water shows a wide variation in different seasons. The pattern of seasonal chlorinity variation in this inlet is shown in Figure 1, data having been obtained from daily measurements on chlorinity in the coastal observation. Figure 1 shows that from late spring to middle winter the chlorinity varied between the approximate limits of 0.5 and 1.5% Cl; but after this period to early spring it approaches nearly zero. It is conceivable that the above pattern of chlorinity variation has a similar tendency at the innermost parts of the inlet. Change of chlorinity in bottom water forms a controlling factor in the survival of the animal habitant, in this inlet, associated with other environmental factors. Especially, it possesses more high importance than the others because of its wide and violent variation. The brackish-water habitat is chracterized by marked salinity variation, and in attempting to analyse the physiological adaptations of brackish-water

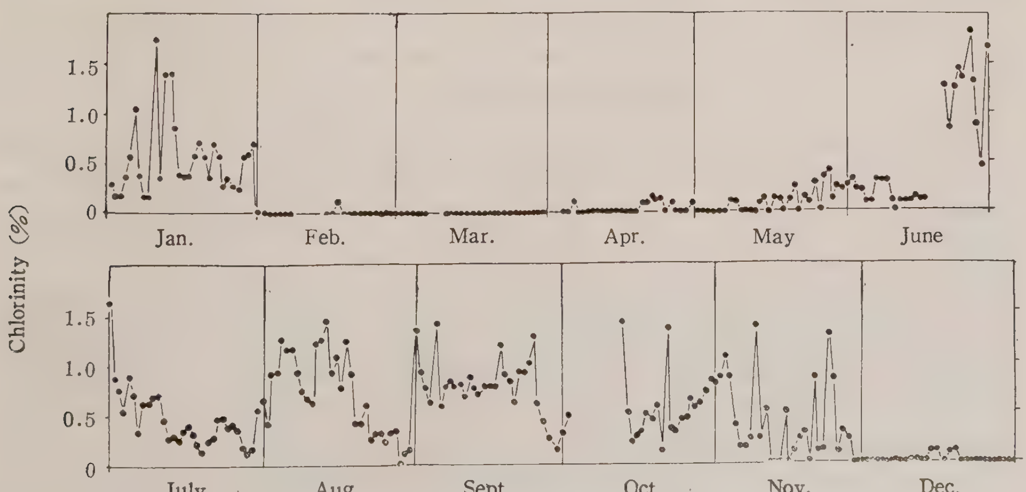


Fig. 1. Pattern of annual chlorinity variation in Zyusan-gata Inlet

animals to their habitat, it is neccessary to consider the extent of salinity variation, which varies from over 1.5% Cl down to nearly fresh water.

It has been pointed out by many researchers that the salinity change in surrounding water exerts physiological and ecological influence on the marine and fresh water animals. In marine bivalves, Fox (1941) demonstrated that the adult California mussel was able to adjust itself to a considerable range of salinities; Hopkins (1936) reported influences of heterosmotic environments on the shell movements and water filtration of Japanese oyster. In brackish-water animals, Beadle (1931) investigated changes in water content and respiratory activity of *Nereis diversicolor* and *N. cultrifera*. Nomura (1930) pointed out that the interstitial salinity in bottom material plays a controlling role with the animal habitants.

The present study was designed to determine the physiological influence of chlorinity change on the clam from the ecological viewpoint. In this paper are reported the results of field and experimental observations showing variability in the shell movements, water filtration and tissue chloride of the clam in responce to the widely differing chlorinity conditions in company with chloride variations in overlying water and interstitial water in Zyusan-gata Inlet.

Before proceeding further, the author wishes to express his cordial thanks to Prof. T. Tamura of the Faculty of Fisheries, Hokkaido University, for his guidance throughout the work. The author is also indebted to Assist. Prof. H. Ohmi of the same Faculty, for his many valuable advices.

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II. Field observations on the chloride contents in bottom water, interstitial water and clam tissues

In the brackish lakes or estuaries, Asahina (1942), Nomura *et al*. (1955) and Smith (1955, 1956) have specified that the chloride concentration of interstitial water in bottom material has an important function as a controlling factor on the survival of benthic animals.

It is said that the different particle compositions of bottom material make a difference in the interstitial chlorinity profiles, and so, in the muddy bottom, even if the chloride concentration in bottom water is suddenly changed in some degree, the interstitial chlorinity is still kept in its initial concentration, while in the sandy bottom —— such as the clam fields in Zyusan-gata Inlet —— it is unstable (Asahina, 1942).

Field observations were carried out to survey the correlation of chloride concentration between bottom water, interstitial water and clam tissues in June, September and

December. Samplings were repeated four times per area in each month. In order to observe the chlorinity profiles th following operations were carried out at the designed six areas. Water from bottm layer at 1 cm above the bottom was sampled by a syringe of about 25 ml capacity and the chlorinity was determined by means of Mohr or Vorhard method. The bottom material was collected in tubular forms by a steel tube, and was divided into four parts of 0–3, 3–6, 6–9 and 9–12 cm in depth. The bottom samples in each part were brought to laboratory in a rubber stoppered glass tube. In the laboratory 30–40 gram samples were spooned out from each tube into small beakers and weighed. Samples were then dried to constant weight in an electric oven at 105±3°C. The loss of weight represents the water contents of each sample. Into each beaker was pipetted 50 ml of distilled water, each dried sample was crushed with a glass rod, the samples were covered to prevent evaporation, and were each beaker were removed 15 ml of supernatant water by a pipetts, and the samples were titrated with silver nitrate. From the chloride value obtained on the 15 ml and the original water content as obtained by drying, the chloride contents of each bottom sample were calculated.

The flesh of clams was prepared for chloride analysis in the following way. The clam was removed from its valves as rapidly as possible. The flesh was blotted on absorbent paper to remove most of the adhering water, then rinsed briefly in 95% alcohol in order to remove most of the remaining water from body surface and gill capillaries. After weighing, chloride analysis of the whole tissues was done by the method of Sunderman & Williams (1931, 1933).

The result of field observations was summarized in Figure 2. In June and September the chloride concentrations in bottom water were higher than that in interstitial water in the surface layer of substratum, while in December they were vice versa in the areas which are situated in the vicinity of a narrow strait connecting with the Japan Sea (stations of Nakazima, Okinose and Shidoshomae); but in the estuarine areas (stations of Yamada, Iwaki and Imaizumi) the chloride in interstitial water had a lower content than that in overlying water in each season.

As shown in Figure 1, the chlorinity of bottom water is nearly zero during winter to spring, but between summer and autumn it has relatively higher value. Accordingly, it may be considered that the interstitial water in surface layer of bottom material has still continued to hold a comparatively high content of chloride. Although the variation of interstitial chlorinity is influenced not only by potentiality of permeation but also by degree of difference in chlorinity between the overlying water and interstitial water, in regard to such variation it may be assumed that under conditions of seasonal variation in the chlorinity of bottom water at each of the given points in the inlet the chlorinity of the interstitial water would be gradually shifted in corresponding fashion. The value of interstitial chlorinity indicates a seasonal variation in the surface layer of

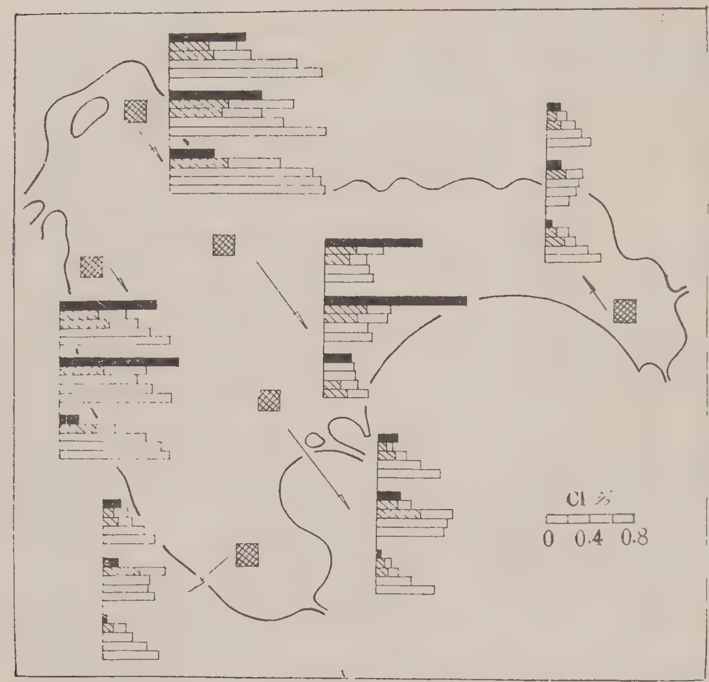


Fig. 2. Comparison of chlorinity profiles in bottom water, interstitial water and clam tissue

In this figure, the histograms of black area (■), white area (□), and shadowed area (▨) indicate the chloride content in bottom water, interstitial water and clam tissue respectively. The three groups of histograms in each areas, indicate downwards the results of chloride profiles in June, September and December respectively, and in each group the interstitial chlorinity profile is downwards divided into four parts, each 3 cm in depth. Each histogram indicates the average value of four times analysis.

bottom material, while 9–12 cm layer in each area the value of chloride content shows a stable situation with no significant seasonal shift. It is strongly suggested that, in relatively deeper layer (9–12 cm depth), the value of interstitial chlorinity may arrive at a nearly stable level because of poor permeation or of secretion of chloride between the overlying water and interstitial water. Although there can be seen a relationship to some extent between chloride contents of clam tissues and that of overlying water in each area and season, no parallel values were seemingly observable. And it is a very interesting phenomenon that the ratio between grams Cl per 100 grams wet tissues and per 100 ml interstitial water maintain an approximate value of 1:2.

From the above field observations is derived the following suggestion. The chloride variation in clam tissues is more strongly affected by the interstitial chlorinity which resulted from the chloride exchange between the overlying water and interstitial water, although it may be directly influenced by the chloride of overlying water to some degree. At any rate, it is obvious that the chlorinity of interstitial water reflects in some degree the chlorinity of the water that has lain above the bottom; the profiles of the interstitial chlorinity were slowly shifted with seasonal variation of the chlorinity of the overlying water, and the tissue chloride of clams varies within a considerable range in proportion to the chloride contents of the external medium in each area.

III. Change in tissue chloride of clam in response to varying salinities

The results obtained from the above field observations have revealed some unknown items of information on the progreess of chloride variation of clam tissue under widely differing salinity conditions. In the following experiments, when chloride concentration of the environmental water is prepared with a sudden and gradual change, the progress of the chloride variation of clam tissues is traced at each designed period.

Experiments were not begun until the clam had been kept in the aquaria for at least two days, under the condition of constant illumination by lighting with a 100 watt tungsten lamp. Most of the clams were 18–22 mm in shell height. Both sexes were employed. In all aquaria the water was continuously circulated and aerated by streams of air bubbles. Dilute sea water was prepared by mixing well water (0.03–0.02% Cl) with stock sea water (1.8% Cl). The water temperature was adjusted at 18±0.5°C by means of a thermostat.

(1) *Change in tissue chloride following sudden alteration in dilute sea water of varying chloride concentrations*

For alterations of expeimental water the fresh water in aquaria is removed at a known quantities and equal quantities of sea water is added without any disturbance on the clam. The added sea water is sufficiently mixed by air bubbles within a few minutes, then it showed the designed chloride concentrations. Tissue chloride measurement was made on groups of five or seven clams taken as a unit in each designed period.

The course of adjustment is presented in Figure 3. Each vertical bar in the figure represents the standard error of four or five determinations. In dilute sea water of 0.4 and 0.8% Cl the chloride content of the clam tissue increases rapidly within the first 8–12 hours. After this period the tissue chloride shows little variation throughout the 150 hours of the experiment, and the tissue chloride represents approximately 0.2 and 0.4% Cl in wet tissues respectively. All animals which were kept in 0.4 and 0.8% Cl for 150 hours were opening their valves, projecting both their siphons and thus were assumed to be normal, but none of the clams in the water containing 1.1 and 1.4% Cl appeared normal. Gradual increment of tissue chloride continues throughout the 70 hours or more in dilute sea water of 1.1 and 1.4% Cl; this phenomenon is detected more distinctly in 1.4% Cl than in 1.1% Cl water.

On the other hand, in fresh water the tissue chloride has no significant variation being maintained in 0.028 to 0.018% Cl throughout 150 hours of the experiment.

A second step of experiments was run to determine the variation of tissue chloride in fresh water after 150 hours in various degrees of dilute sea water. The curve of change in chloride content is plotted on the right side of Figure 3. During the first 4

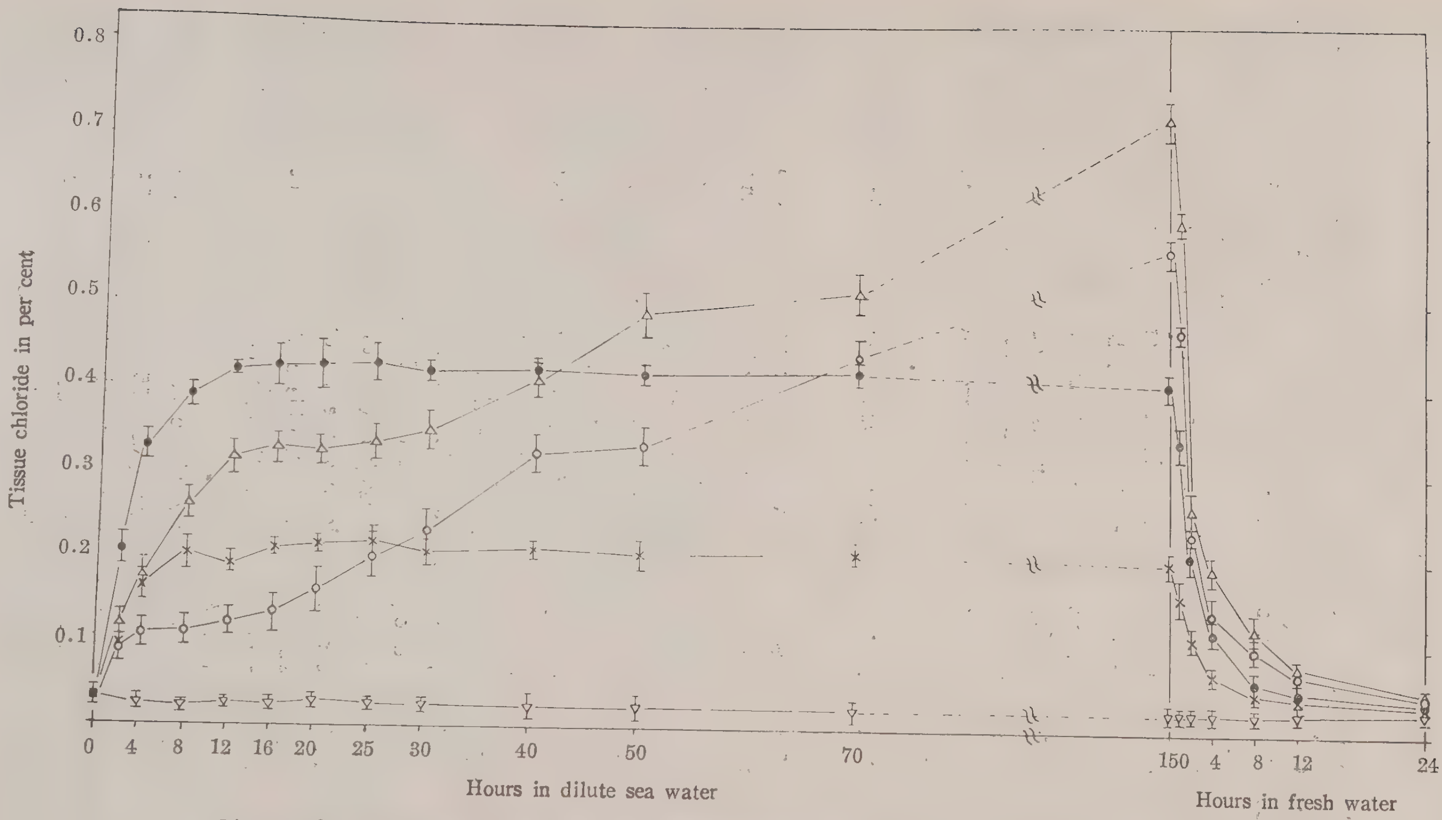


Fig. 3. Changes in tissue chloride of clam following sudden alteration of chlorinity in surrounding water. Vertical lines indicates plus or minus of the standard error of the mean. The symbols indicate as follows: ▽—▽ fresh water (0.028% Cl), ×—× 0.4% Cl, ●—● 0.8% Cl, ○—○ 1.1% Cl, △—△ 1.4% Cl.

hours this value decreases quickly. After this period the gradual loss of chloride continues until 24 hours or more, whenafter the chloride content varies 0.028–0.042% Cl. The chloride loss continues with approximately the same tendency in every case, although the decrease in tissue chloride is slightly rapid in lower content as compared with that in the higher one.

(2) *Chloride variation shown by the clam in gradually altered chlorinity of environmental water*

The gradual alteration of the environmental water was brought about in the following manner. From an aquarium a known quantity of fresh water was withdrawn, discarded and replaced by an equal quantity of sea water without any disturbance to the clams at 12 hour intervals. These alterations brought about a gradual rise from the initial value to 0.2% Cl in the next solution, during the experimental period. When the experimental solution attained to 1.8% Cl, above treatments for gradual rise of the water were performed in opposite direction until the solution returned to fresh water condition.

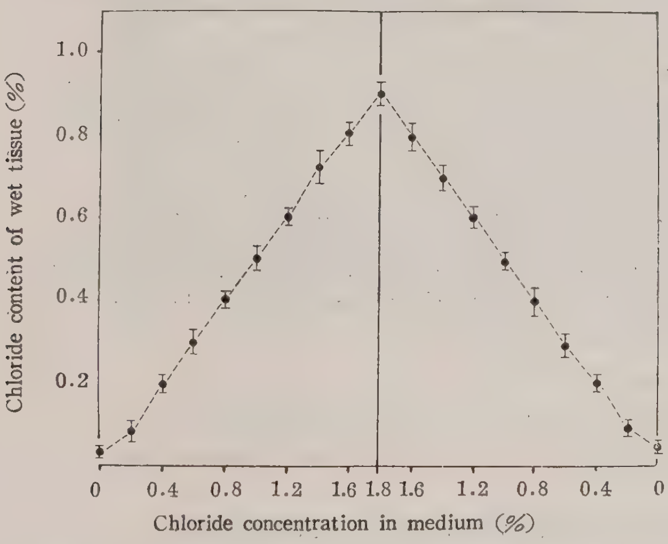


Fig. 4. Chloride variation of the clam tissue in gradually altered chlorinity of environmental water
Vertical lines cover the range of the standard error of the mean.

Figure 4 shows the results of chloride analysis. It is clearly illustrated from the foregoing experiments that the maintenance of an approximately constant ratio of 2:1 occurs between the chloride concentration in the surrounding media and the tissue chloride of clam immersed therein as is noted by the straight line between nearly zero and 1.8% Cl.

According to Fox (1941) the tissue chloride of the adult California mussel shows no significant difference under the constant chloride conditions all the year round; and mature males show slightly higher chloride contents than the mature females do. Although no attempts were made to confirm these facts in the clam, however, it will be of no significance in respect to the purpose of this work.

IV. Relation between chloride concentrations of experimental media and physiological activity of clams immersed therein

In lamellibranch mollusks the process of feeding is regulated by three factors: function of the ctenidial cilia, activity of the adductor muscles attached to shells and openness of inhalent siphon or pore. It is easily presupposed that the variation of tissue chloride may cause some signs of disturbance in th physiological activity of the animals. The following experiments were made to determine how the physiological activity changes with the chloride variation of clam tissue. In this experiment as an available measuring method of physiological activity use was made of shell movements and water filtration, which reflected all factors which may influence the calms. The experimental conditions, such as illumination, pH and temperature, were similarly adjusted as described in the former section.

(1) *Shell movements of clams in various degrees of dilute sea water*

Records of shell movements were made on smoked paper of clock-work kymograph which were geared to make one revolution in 24 hours. For an analysis of the recordings, the area between the record line and the base line (complete closure) were calcuated by recording on section paper. These experiments were repeated on six individuals in each designed dilute sea water. Response was expressed by the percentage between the total area enclosed per 12 hours in the fresh water and that in each designed solution. The term "shell movements" indicates only the actual movements of the shells and the distance apart of valves is expressed as "degree of openness".

Typical records are shown in Figure 5. In general, from the kymographic records the shell movements of clams may be represented by five shell positions or forms in accord with the increment of chloride concentration ranging from fresh water to 1.8% Cl. The five forms can be classified as follows:

- Form I : Shell movements rhythmical and regular, complete opening
- Form II : Shell movements more rhythmical and violent, temporary increment of openness
- Form III : Shell movements irregular, gradual diminution of openness
- Form IV : Stationary or occasional shell movements, but opening
- Form V : No shell movements, complete closure or narrow opening of the shell for almost entire duration of the experiments

When the experimental solutions are immediately converted from fresh water to salt water, in 0.4% Cl, shell movement tracing showed Form II during first 1 or 2 hours, but after this period the movement returned to Form I. In 0.8 and 0.95% Cl the shape of movements represented Form II and was followed by Form III; after between 5 and 10 hours it then returned to Form I. Nevertheless, in the dilute sea water of 1.1% Cl

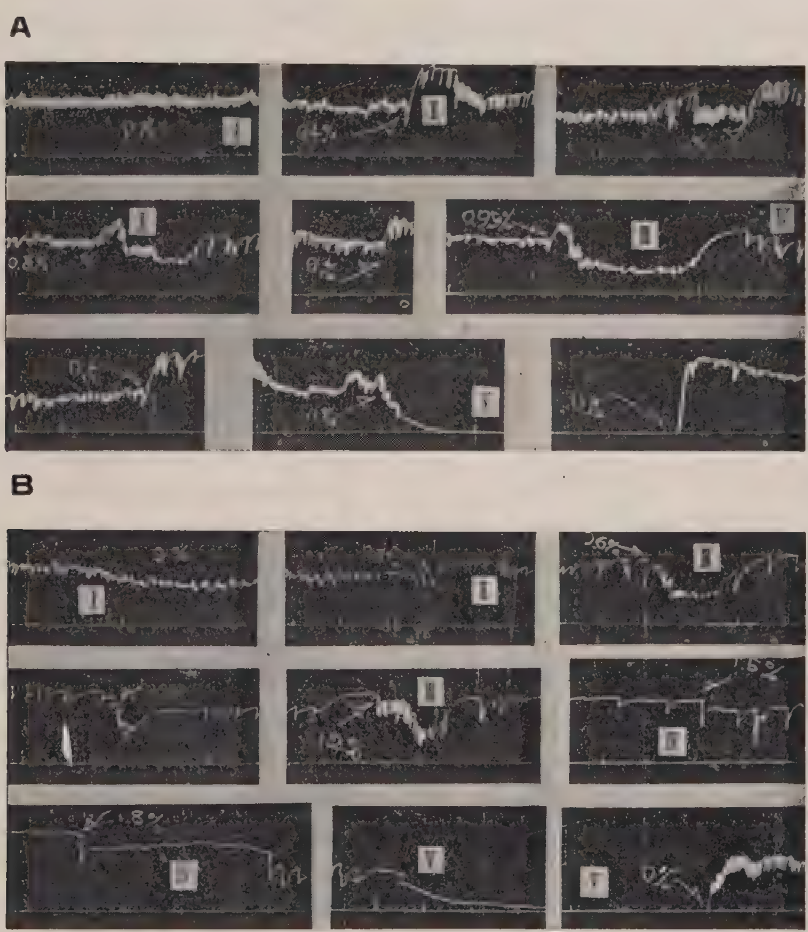


Fig. 5. Typical records of "shell movements" of clam when the environmental water increased suddenly (A) and gradually (B) from fresh water to various dilutions of sea water

Roman numbers in this figure indicate five shell movement forms. Time signals on the base line (complete closure) are marked at one hour intervals.

the addctor muscle delineated furious movements following the change of environmental solution from fresh water; the gradual decrease of openness (Form III) continued a few hours, finally arrived at Form V. Such tendency become more evident in 1.4% Cl.

On the other hand, when the chloride concentration of solution varying between fresh water and nearly straight sea water (1.8% Cl) was gradually increased, shell movements of clam showed a considerable difference. The typical examples of shell movements are shown in Figure 5 B. No significant differences were traced between the shell movements in 0.3% Cl and those in fresh water, while in 0.6, 0.9 and 1.2% Cl shell movements drew Form II, followed by Form III. In these concentrations the duration of Form III

activity was prolonged with an augmentation of chloride contents in the experimental solution. In 1.5% Cl, within first 3 hours the shell movements showed Form IV, and in 1.8% Cl there appeared no movements, narrow opening or complete closure of the shell (Form V) during same period. When the experimental media were suddenly converted to fresh water, gradual gain of the openness continued during the first few hours in company with violent movements, and then position arrived at Form I.

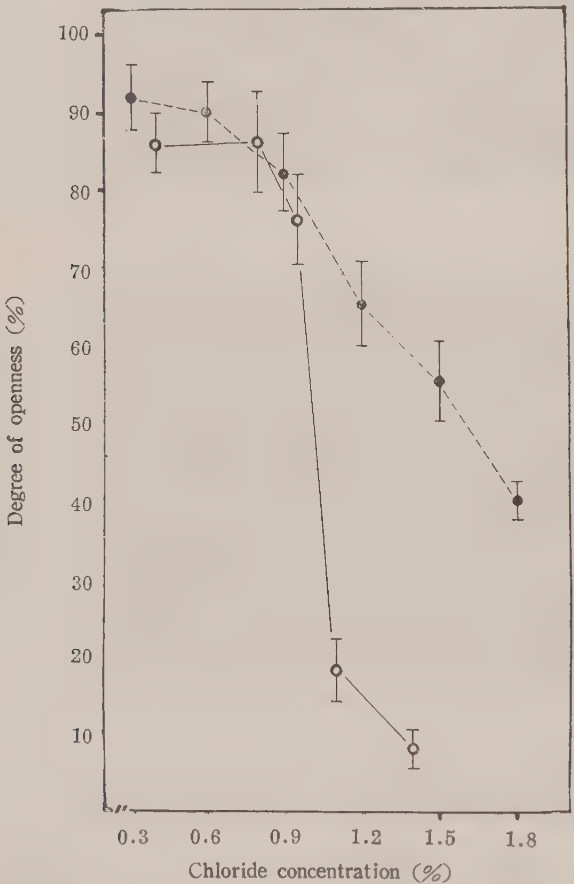


Fig. 6. Showing the "degree of openness" of valves in various chlorinity

Full line represents the values in case of sudden increase in various chlorinities, and broken line represents gradual increase. The vertical lines indicate plus or minus of the standard error of the mean.

The results of analysis of the kymograph recordings is summarized in Figure 6. In chloride contents between fresh water and 1.0% Cl, a comparison between the degree of openness in sudden rise of the chloride concentration and that in gradual rise, reveals that the similarities between the two cases are more striking than the differences and there is no significant difference, while in above 1.1% Cl concentration the difference is significant, the former more abrupt decrease of openness than the latter.

(2) *Change of water filtration in various dilution sea water*

In this experiment the water filtration of clam was measured by the rate of removal of particles in suspension of acid earth. Stock suspension was prepared by following treatments. Seven grams acid earth was added to one liter fresh water, the sample was mixed by stirring, and was allowed to stand for 2.5 hours, finally the suspension of acid earth was decanted without any disturbance of the precipitates. Suspension in experimental water was prepared from the stock fresh water suspension. A dilution of one part of stock suspension with ten parts of salt water was used. Such preparations have an approximately 90 mg weight per liter

in initial concentrations of the acid earth, and the particles of most usual size were 2–6μ in diameter. In the experimental solution containing various chloride concentrations, the acid earth was mixed by means of continuous air bubbles for aeration to form a stable suspension within at least the first 3 hours. During the experiments pH values varied betwwen 7.0 and 8.3. Measuring of water filtration on groups of 17 adult clams (18–22 mm in shell height) taken as a unit began after 12 hours in the water of known chloride contents.

The rate of filtration was computed from the formula given by Jϕrgensen (1949). This is

$$P_t = P_o \quad exp \left[- \frac{M}{m} \cdot t \right]$$

in which M is the value of suspension in liters, m is the quantity of water filtered in liters per hour, while P_o and P_t are the concentrations of the suspended material at the initial time and after t hours respectively. The concentrations of acid earth were determined colorimetrically by means of a photoelectric colorimeter. It was found that the light absorption, expressed as a function of the concentrations of acid earth, followed Beer's law. In these experiments, therefore, the proportion of particles removed from acid earth suspension could be directly determined from the corresponding decrease in light absorption.

The average values of water filtration per individual adult clams per hour are shown in Figure 7. Difference between the average of the filtration in case of sudden rise of salinity and that in gradual rise is not recognized, the augmentation of pumping is measured by the corresponding increase of chloride concentration ranging between fresh water and 0.8% Cl. In the chloride concentrations above 1.0%, an average value of filtration in sudden

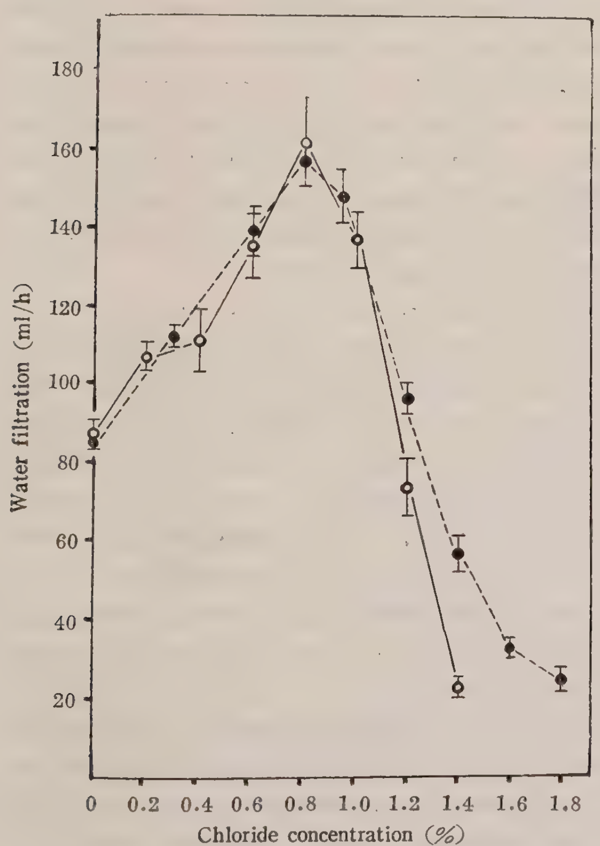


Fig. 7. Average values of water filtration in various dilution sea waters
Each symbol and vertical bar is the same as those in Figure 7.

rise is slightly inferior to that in gradual rise, but the difference is significant.

From these obtained results, it is suggested that the influences on salinities are much stronger upon the function of ctenidial cilia than on that of adductor muscle, in chlorinity of under 1.1%. On the other hand, in the solution above 1.1% Cl, the rhythmical movements of shell decline, and diminution of openness begins with this concentration. Moreover, decrease of water filtration is conspicuously displayed under such chloride concentrations. In conclusion, from these experiements it may be stated that several signs of disturbance in physiological activity of the clam were caused in water of chloride contents above 1.1% Cl.

V. Discussion

The factors which influence the physiological activity of clams, for example, Cl regulation, shell movements and water filtration can be stated to be such physico-chemical conditions as pH value, water temperature and light intensity with the exception of salinity. Recently, in the freshwater mussel, *Hyridella australis*, Hiscock (1950) has reported that within temperature range 17 to 27°C, there was evidenced no well-defined influence on shell movements or on the degree of openness, whereas, the light intensity showed a marked influence. In the present study, water temperature and light intensity were constantly adjusted during the course of experimets, and as shown in Table 1, variation of tissue chloride, degree of openness and water filtration have been recognized to be insignificant within the pH range of 7.0–8.3.

The variations in tissue chloride may have resulted not only from the permeation or secretion of salts, but also from the loss or gain of water. If such chloride exchange proceeds throughout all the semi-permeable tissue of clams, it is a very interesting phenomenon that the maintenance of a ratio of roughly 1:2 between grams per 100 gram wet tissues and grams Cl per 100 ml salt water is noted as a straight line within the chlorinity range of 0.03 and 1.8%, and that in this respect it is similar to the result obtained in California mussel by Fox (1941). Although none of these experiments deals with the actual mechanism involved in osmo-regulation and physiological activity of clams, the maintenance of a constant ratio between internal and external chloride may result from neural activities which have some means of controlling movement of water and salt through the semi-permeable tissue of clam.

In view of the fact that the duration of remarkable increment of tissue chloride after change from fresh water to sea water coincides with the duration showing the pattern of shell positions of Form II or III in weaker chloride water (0.3–0.9% Cl), the violent movements of shell recorded are reasonably presumed to be the result of Cl exchange between internal and external water.

In summarizing the above field and experimental observations, it is recalled that the adult clam is able to adjust itself, with accompanying changes in tissue chloride, to a

Table 1. Influence of different pH value upon tissue chloride and physiological activity of clams in dilute sea water
In the course of the experiments, water temperature is 18±0.5°C and chlorinity of water is adjusted in 0.4% Cl.

(A) Tissue chloride

pH	Chloride contents (% Cl)			
	0 hour	4 hours	12 hours	24 hours
7.0	0.039	0.125	0.190	0.194
	0.032	0.128	0.189	0.210
	0.040	0.133	0.211	0.193
	0.037	0.128	0.197	0.199
	0.034	0.119	0.197	0.200
8.3	0.040	0.115	0.161	0.193
	0.040	0.145	0.152	0.194
	0.037	0.126	0.166	0.202
	0.039	0.129	0.181	0.196
	0.034	0.119	0.197	0.203

$F_0 = 1.46 < F_{(0.05)} = 216$ (insignificant)

(B) Degree of openness

pH	Openness (mm^2)					
	1st	2nd	3rd	4th	5th	6th
7.0	1660	1320	1570	1880	1490	1590
8.3	1710	1220	1510	1800	1550	1470

$F_0 = 1.55 < F_{(0.05)} = 6.61$ (insignificant)

(C) Water filtration

pH	Filtration (ml/h)			
	1st	2nd	3rd	4th
7.0	142	96	121	117
8.3	131	112	90	121

$F_0 = 1.46 < F_{(0.05)} = 216$ (insignificant)

considerable range of salinities in its habitats in Zyusan-gata Inlet as well as laboratory. Accordingly, even if this animal were exposed to a foreign environment, suddenly changing from fresh water to sea water, it may tolerate the change for a considerable period in the extreme case. Such euryhalinous character of this animal may result not only from the adaptability of osmo-regulation furnished by itself, but also from the function of interstitial water which mollifies any sudden change of salinity.

It is indubitable that the salinity variation in surrounding water is not the only factor responsible for the physiological activity of *Corbicula japonica*, but it may be

suggested that the influence of salinity change upon the sensitivity of adult and larval clam provides one helpful clue in further investigation on various physiological and ecological problems.

VI. Summary

1. The results of field observation indicate that the chloride variations of clam tissue were affected more strongly by the interstitial chloride in bottom material than by the direct influence of the overlying water.

2. When the experimental water was changed suddenly from fresh water to various degrees of dilute sea water, the tissue chloride showed significant changes in laboratory experiments. The chloride increased markedly during the first 8–12 hours, but after this period the values of tissue chloride attained to a constant level showing an approximate value of 1:2 in ratio between grams Cl per 100 grams wet clam tissue and per 100 ml environmental water, in 1.1% Cl or under, however, in the chloride concentrations above 1.1% Cl, the chloride variation showed a considerable difference as compared with above example, and the gradual increment of tissue chloride continued during the period of 150 hours.

3. If chloride content is gradually changed, a roughly constant ratio of 1:2 is demonstrated between the tissue chloride of clam and the chloride content in surrouding media ranging from nearly zero to 1.8% Cl.

4. Shell movements and water filtration of clams were adopted as an available method for measuring their physiological activity. From these experiments, it is suggested that several signs of disturbance in physiological activity of the clam were caused under the condition of chloride concentrations above 1.1 or 1.2%.

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GROWTH AND BREEDING SEASON OF THE BRACKISH-WATER BIVALVE, *CORBICULA JAPONICA*, IN ZYUSAN-GATA INLET

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In some lamellibranchial bivalves, as a sign of their growth, the annual rings were shown on the surface of their valves, transverse section of shells and adductor muscle scars, and these are helpful in determining the age and growth rate of the clam. It is believed that these marks are made in any stagnating seasons of conchiolin secretive ability in the mantle margins. According to this supposition, the determination of age and growth rate in many marine bivalves have been studied by means of the shell reading method (Orton, 1926 ; Stephen, 1929 ; Yamamoto, 1947 ; Coe, 1947 ; Nayar, 1955 ; Cassei, 1955). In bivalves which have no distinct growth marks, size frequency distribution has been often employed for the presumption of age and growth rate. On *Corbicula sandai*, Furukawa (1953) has presumed age and growth rate by the shell reading method, however, in *Corbicula japonica* no growth marks, as concentric circular resting zones, appear on the surface of their valves and other parts, and information available on the growth of this economically important clam is scanty at present.

This study was initiated in order to gain some information on the growth rate of *Corbicula japonica* in natural population, and in addition such information was used as a presumption on the breeding seasons of the clam in Zyusan-gata Inlet.

The author wishes to express his cordial gratitude to Prof. T. Tamura of the Faculty of Fisheries, Hokkaido University, for his constant guidance in the course of the work. Thanks are also due to Assist. Prof. H. Ohmi of the same Faculty who gave many valuable suggestions.

Method

Random sampling of the clam, *Corbicula japonica*, was made once a month for the period from June 1954 to October 1955, from various main clam fields (viz., Okinose, Est. Iwaki, Est. Yamada and Est. Imaizumi) in Zyusan-gata Inlet. All animals were sampled every time by means of a hand dredge with sieves of 1 mm square mesh.

Growth rate is determined by the size frequency method. Thus, as the standard of measuring size, shell height was measured with sliding calipers on a unit of one tenth of a mm and measurements were grouped into sizes according to 1 mm intervals. Then the percentages of different size groups to the total number of clams measured for each month were represented by graphs. The polymodal frequency distributions obtained by observation were graphically analysed making use of probability graph paper devised by Harding (1949). Each normal distribution, which was separated from the polymodal frequency distribution, was compared with ordinal frequency under the statistical

treatment of chi-square test, and was tested for the goodness of fit. The shifting of the mean values in each normal distribution for different months was taken to indicates the average growth rate of the different year-classes from month to month.

The spawning period of the clam was presumed from monthly size frequency distribution and from determining the annual variations in nutrition and meat weight coefficient of the clams. The size of clams used in this analysis was 18 mm or more in shell height. The valves were carefully opened without any damage to flesh, most of the adhering water in body surface and gill capillaries was removed by absorbent papers, then each clam was weighed with and without the valves. The meat weight coefficient of the clams was derived following formula;

$$\frac{\text{wet weight without valves}}{\text{total wet weight with valves}} = \text{meat weight coefficient of clam}$$

The flesh was dried to constant weight in an electriç oven at $105 \pm 3°C$; the loss of weight represented the water contents of each sample. Total nitrogen in dried sample was analysed by Kjeldahl method.

Water filtration was applied as an available index of physiogical activity of the clams. As shown in a previous paper (Fuji, 1957), the removal of particles from the suspension of acid earth was measured by the optical density of photoelectric colorimeter, and water filtration was calculated from the formula derived by Jørgensen (1949). In the experiments of water filtration, all aquaria were continuously aerated with air bubbles, and experimental water was adjusted to 0.6% Cl.

Result and Discussion

The size frequency distribution in each mm interval is represented in Figure 1. From this figure, it is pointed out that three or four size groups were separated from the frequency distribution in each month. In the month of July 1954 the samples observed consisted of four size groups: the mean value of the first group, in which it is assumed the larval population is only shifting in bottom life, is estimated at 2.5 mm; in the second, the third and the fourth groups the mean value of each separate group is appraised at 8.4, 15.5 and 21.2 mm respectively. The shifts of these mean values of each size group, as given in Figure 1, increase steadily with the progress of months. For example, in the second group the gradual and steady increment of mean value is represented by the movement from 8.4 mm to 10.3, 11.2. 11.7, 11.8 and 12.0 mm in successive months. In July 1955 the mean values of four size groups separated were represented at 2.1, 7.5, 14.7 and 21.0 mm in shell height, and these values coincide with the one in the month of July 1954. From the results of above observations, it is strongly suggested that the three or four different size groups separated from the frequency distribution correspond with each different age-class. If this presumption is admitted, it may be concluded that the values of each different age class demonstrated

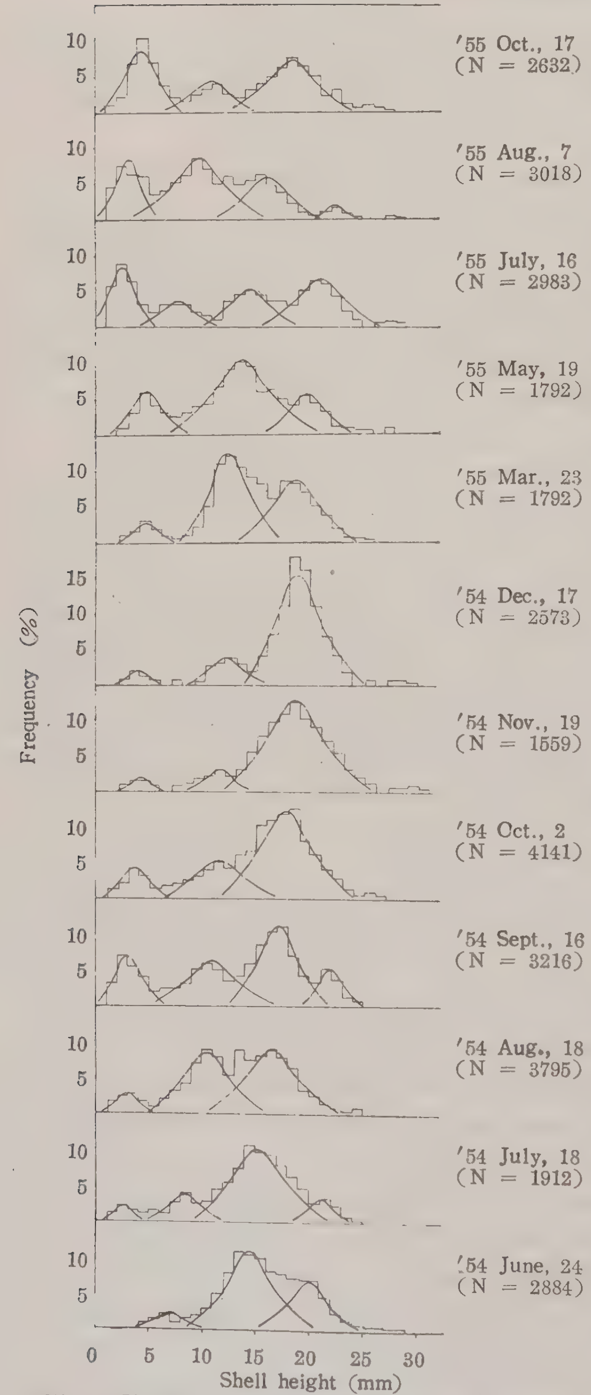


Fig. 1. Shell height frequencies of the clam, *Corbicula japonica*, caught in Zyusan-gata Inlet

Histogram : Shell height frequency distribution obtained by observations. Curve : Shell height groups estimated by fitting normal curves.

an approximate value of 9,15 and 21 mm in shell height at the ages of full 1, 2 and 3 years.

The correlation between the growth and the temperature in successive months is represented in Figure 2. The growth of the clam started in April when water temperature attained to approximately 10°C, a rapid increase of growth showed in May (temperature about 15°C) and in the season between June and August showing temperature of over 25°C, it was very vigorous. Their growth continues until November when the temperature was about 5°C, although in September and October the degree of increment in growth falls off. However, it is distinct that the augmentation of shell height ceases in the season between December and March.

The fact that the growth and breeding in aquatic animals are influenced to a considerable extent by fluctuations of environmental factors such as water temperature, salinity and others has been well recognized. Especially, in the shallow waters, in such clam fields as in Zyusan-gata Inlet, water temperature changes markedly between summer and winter resulting in rapid growth of clam shell height in the former season and less or negligible growth in the latter. In many marine bivalves, Orton (1926) on *Cardium*

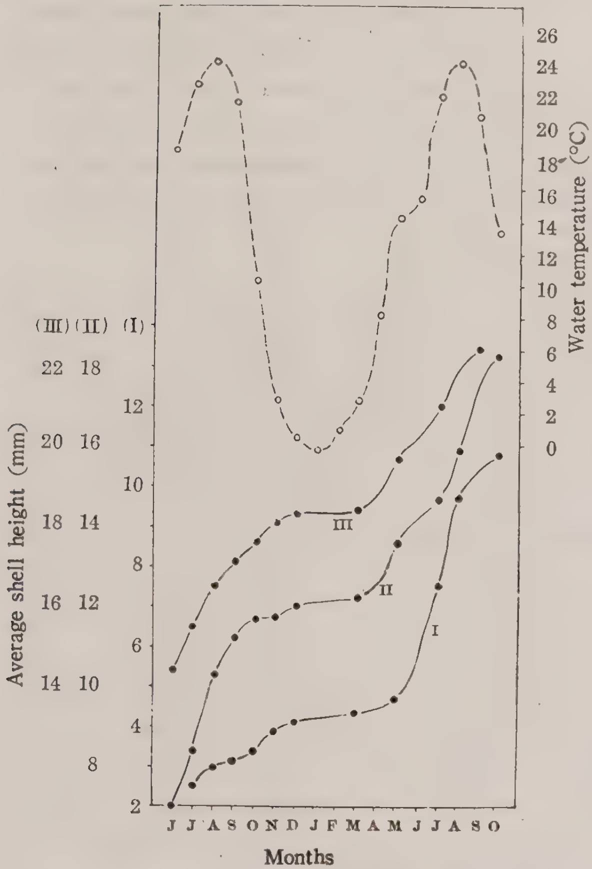


Fig. 2. Diagram showing the average growth rate of clams belonging to each year-class with water temperature in successive months

edule, Naito (1930) on *Mertirix mertrix* and Yamamoto (1947) on *Mactra sachalinensis* have furnished detailed information on such tendency of growth rate. It is believed that such annual variation of growth rate is influenced by the physiological activity of the clam resulting from the seasonal cycle of water temperature.

The influences of temperature on water filtration which is useful as an available measurement of physiological activity are given in Figure 3. From inspection of this figure, it can be stated that the value of water filtration under various temperatures increases linearly with the rise of temperature. In view of the fact that the measurement of filtration rate gives a reliable measure of current flow, it may be suggested that the feeding rate of the creature in higher temperatures shows more activity than in lower one, and the rate is closely comparable with the seasonal vicissitudes of growth rate.

In the monthly size frequency distribution (shown in Figure 1), a fresh year-class has commenced entering the population is revealed by the appearance of very small individuals of 1 to 2 mm height in mid-July of both years, and the occurence of the major set larval population is found still in September. A margin of about 2 to 3 weeks is allowed for embryonic, larval life, setting and growing to 1 or 2 mm in height, moreover, the most of samples used to prepare the size frequency distribution consisted of various sized clams left in the sieve of 1 mm square mesh. Consequently, it is assumed that the occurring period of set larvae lies in a few weeks before the middle of July.

Variation in tissue contents such as nitrogen, ash and moisture represents an annual cycle influencing the maturity and breeding. Sekine *et al.* (1929) on oyster, Ashikaga

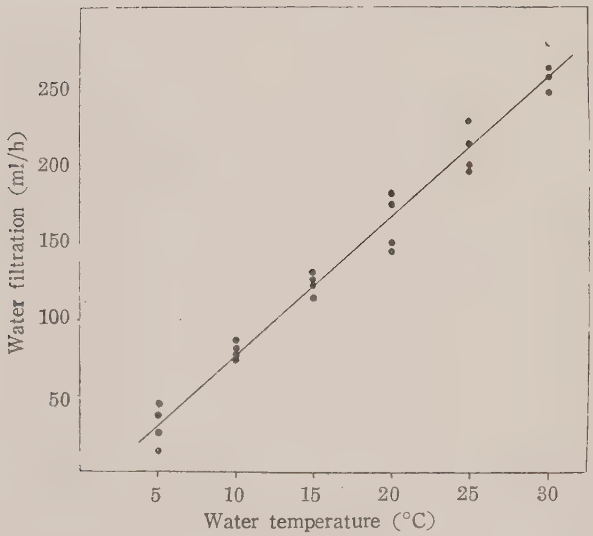


Fig. 3. Relation between water filtration of clam and water temperature

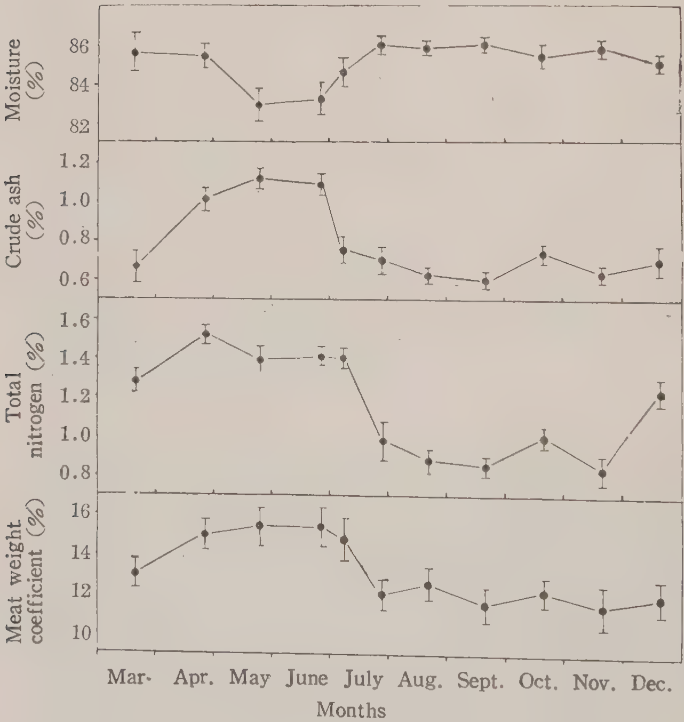


Fig. 4. Seasonal variation in the flesh components of clam (per cent in row material)
In this figure the vertical lines indicate a range of standard error of mean.

(1948) on pearl-oyster have reported detailed information in relation to the above problems. The samples obtained regularly by present author once a month during ten months between March 1955 and December 1955 were analysed in order to determine the annual variation in nitrogen, ash, moisture and mest weight coefficient of the adult clam (18–22 mm in shell height). The results are plotted in Figure 4. The peak values of meat weight coefficient, nitrogen and ash contents were attained during late May and late June, but in early and middle July they decreased strongly. On the other hand, moisture showed seasonal variation contrary to other elements. From these results, it may be assumed that the period between late May and middle June corresponds to the most maturating term of this clam and occurs just before the breeding.

According to Asahina (1941), in Mokoto-numa, Hokkaido, almost all

females were spent individuals in middle July. Kokubo & Sato (1947) have reported that the major planktonic larvae of clam were found in plankton samples in August in Zyusan-gata Inlet. In view of these items of information, it is concluded that the breeding seasons of the clam in Zyusan-gata Inlet appear to be prolonged, extending from late August, and the majority of the individuals spawn in early July, although the breeding would be commenced at different times to some extent in various clam fields.

Summary

Monthly distributions of shell height frequency of the clam were analysed using a probability graph paper, covering the period from June 1954 to October 1955. Then they were separated into three or four normal distributions which correspond to the different year-classes. The growth rate of the different year-classes in successive months indicated the shifting of the mean values in each normal distribution for different months.

The growth of the clams in each different year-class commenced in April, and in the summer seasons showing the water temperature over 25°C, the growth was rapid. After this season, the gradual growth continued until November, but in the winter season between November and March the clam showed a less or a negligible growth.

A fresh year-class consisting of the very small individuals of 1 to 2 mm in height commenced entering the population in middle July. Moreover, the nutrient variation of clam tissues demonstrated considerable fluctuations in early and middle July. Thus it is shown from the above information that the breeding season extends from late June to late August, and the majority of individuals spawn in early July.

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チガイソの游走子嚢に於ける核分裂

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Nuclear Division in the Sporangium of *Alaria crassifolia* KJELLM.

Hiroshi YABU

Abstract

The nuclear division in the sporangium of ***Alaria crassifolia*** KJELLM. is described in this paper. This is believed to be the first cytological study in the genus Alaria. In the prophase of the first nuclear division, the typical synapsis, spireme and diakinesis stages were observed (Figs. 7–11), and in the metaphase of the first and successive divisions, 22 chromosomes were counted (Textfig. 1; Figs. 12, 13, 22, 24). As a result of five successive nuclear divisions, 32 zoospores are produced in each sporangium (Fig. 26).

日本産コンブ科植物の游走子形成の際の核分裂については，今日迄に，マコンブ ***Laminaria japonica*** ARESCH.（阿部，1939），ワカメ ***Undaria pinnarifida*** (HARV.) SUR.（猪野・西林，1955），ミツイシコンブ ***L. angustata*** KJELLM.（西林・猪野，1956）およびスジメ ***Costaria costata*** (TURN.) SAUNDERS.（西林・猪野，1957）の４種について研究が発表されている。

今回，これら４種と属を異にするチガイソ ***Alaria crassifolia*** KJELLM. の游走子嚢に於ける核分裂を観察することができたので，ここに報告する。

材料と方法

材料は1956年12月30日と1957年１月中旬の２回，函館市立待岬で採集し，実験室に持ち帰つて胞子葉だけを海水を入れたバットの中に生かしておき，採集した日とその翌日の２日にわたつて固定を行つた。固定には阿部氏液（阿部，1933）を用いた。即ち次の a，b，c ３液を１，１，２の割合に混合した液を使用する。

a）	ピクリン酸飽和液	50cc.
	氷　　醋　　酸	5cc.
	ク　ロ　ム　酸	1gr.
b）	ピクリン酸飽和液	25cc.
	40％ホルマリン	25cc.
	尿　　　　素	0.5gr.
c）	クロム酸貯蔵液（海水98cc，クロム酸飽和水溶液２cc）	50cc.
	２％オスミューム酸	5cc.
	海　　　　水	50cc.
	氷　　醋　　酸	2.5cc.

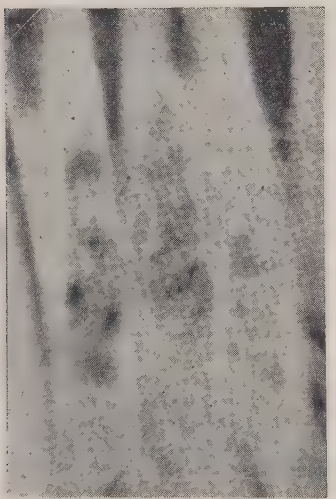
固定時間は８〜12時間とし，プレパラートの作製はパラヒン法により，切片の厚さは３〜４ μ とし，染色はハイデンハイン・ヘマトキシリン法によつた。

観　　察

游走子嚢形成の順序は，西林・猪野（1956）のミツイシコンブでの観察と一致している。即ち，先ず胞子

葉の表皮細胞が表面に平行な膜で2分し，内外2層の細胞となり，外層の細胞は伸長を始め，やがて細長い側糸となり，隣りの側糸との間に間隙ができるようになる。其の後，側糸の基部の細胞，即ち表皮細胞の分裂の結果生じた内層の細胞が，この間隙に向つて隆起し，次いで隔膜ができて隆起部は1個の細胞となる。これが若い游走子嚢で，中心に1個の休止核がみられ，これは1個の仁を持つている（Figs. 1. 2）。

この核は次第に大きさを増し，核内には仁のほかに少量の染色質が現われるようになる。この時期には仁のほかに仁と同じ位の大きさの物体が同時に核内にみられることがある（Figs. 3. 4）。やがて核内の染色質の小粒は糸状となり，次第に太くなり，色素によく染まるようになる。この染色糸は仁と密接して始めは核内の一側部に集まつているが，次第に核腔内に拡がつてくる。この時期には各染色糸の分岐点，末端核膜との附着点等には染色質節が観察される（Figs. 7〜10）。染色糸は形成の始めには仁と，また仁とよく似た構造物とも密着しているのが認められた（Fig. 6）。核腔内一様に拡がつた染色糸はやがて切れて次第に二価染色体になる。diakinesis 期には仁と20個余りの二価染色体が観察された（Fig. 11）。分裂中期には核膜と仁はともに完全に消失し，極面観で22個の減数された染色体がみられ（Textfig. 1; Figs. 12，13），側面観では紡錘体がみられた。稀に両極に中心体らしい小体が観察されたが（Fig. 15），この小体は観察されない場合が多い。後期には染色体群が両極に分れて移動しているのが観察されたが（Fig. 16），紡錘糸は認められなかつた。終期には染色体は見えなくなり，2個の娘核が形成され，核には核膜と仁が再び現われる（Fig. 17）。その後更に4回連続して核分裂が行われ，その結果，游走子嚢内には32個の遊離した核が形成されるが（Figs. 12〜25），各分裂の中期には22個を越えない20個前後の染色体が算えられた。第五回目の核分裂が終る頃から游走子嚢の頂端附近から膜の形成が始められる（Fig. 25）。核分裂が終ると，游走子嚢内には帯色体が次第に数を増し，1個ずつ各々の核に接近した位置をとる。やがて1個の核と1個の帯色体を含む小部分づつを包む薄い膜が形成され，32個の游走子ができあがる（Fig. 26）。



Text-figure 1. Early metaphase in the first mitosis of a zoosporangium. From a microphotograph of a section through a sporophyll of *Alaria crassifolia* KJELLM., showing in the center the same zoosporangium as shown in Plate I, Fig. 13. × 1250.

論 述

コンブ目植物の細胞学的研究は，今迄に前述の日本産の4種のほか，*Chorda filum* (KYLIN '18,)，*Eisenia Menziesii* (MEYERS '28, *Pterygophora Cliafornica* (McKAY '33)及び*Eisenia arborea* (HOLLENBERG '39）について発表されているが，いずれも游走子形成の際に減数分裂が行われることが明らかにされている。本研究においても，チガイソの游走子嚢内の第一回核分裂の diakinesis 期に二価染色体を観察し典型的な減数分裂が行われること，染色体数は n＝22であることを確めることができた。この染色体数は，従来研究されたコンブ目の種類のうち日本産のマコンブ，ミツイシコンブ及びワカメ，ヨーロッパ産の *Laminaria Cloustonii* (WALKER，未発表研究として西林・猪野，1956に引用）で報告された染色体数と一致する。

この研究に当つては材料の固定その他について本学部動物発生学教室の新山英二郎助教授の御指導を得，又，本稿は時田教授の御校閲を辱くしたことを茲に感謝するものである。

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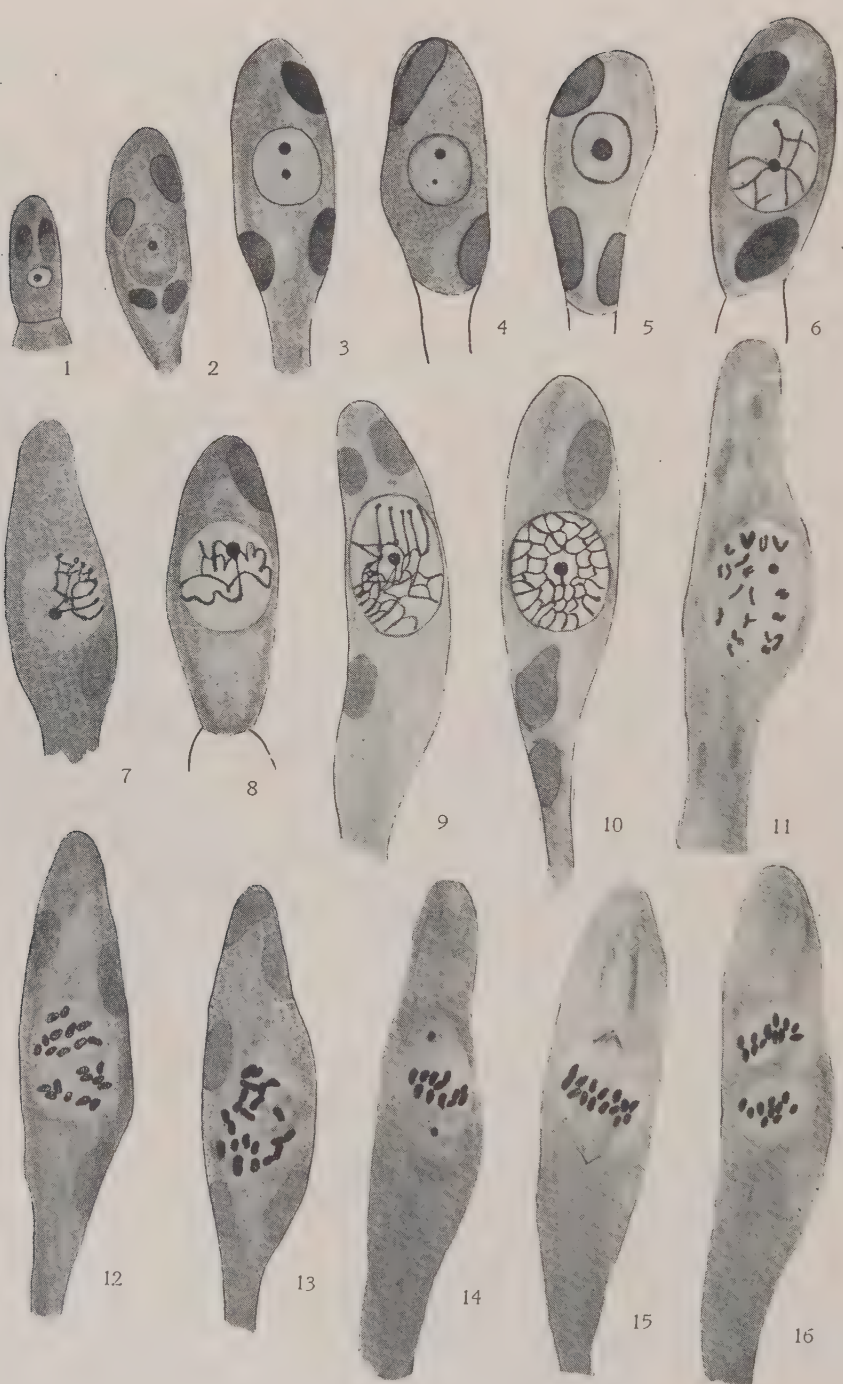
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Legend to Figures in Plates I and II

Figs. 1-26. Nuclear divisions in the zoosporangia of *Alaria crassifolia* Kjellm.

Figs. 1-5. Resting stage. Figs. 6-9. Synapsis. Fig. 10. Spireme. Fig. 11. Diakinesis. Figs. 12-13. Bi-early metaphase. Figs. 14-15. Metaphase. Fig. 16. Anaphase. Fig. 17. Two nucleate stage. Fig. 18. Metaphase of the second division. Fig. 19. Quadri-nucleate stage. Fig. 20. Metaphase of the third division. Fig. 21. Octo-nucleate stage. Fig. 22. Metaphase of the fourth division. Fig. 23. Sixteen nuclei stage. Fig. 24. Metaphase of the fifth division. Fig. 25. Thirty-two nuclei stage. Fig. 26. A fully matured zoosporangium containing zoospores. Magnification: Figs. 1-16, × 2300; Figs. 17-26, × 1290.



H. YABU: Nuclear division in the sporangium of *Alaria crassifolia* KJELLM.



H. YABU : Nuclear division in the sporangium of *Alaria crassifolia* KJELLM.

体長分布よりみたホッケ *Pleurogrammus azonus* JORDAN et METZ の成長

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On the Growth of "Hokke", *Pleurogrammus azonus* JORDAN et METZ, Considered on the Basis of Length Distribution

Ken-ichiro KYUSHIN

Abstruct

In the present papar, an attempt was made to estimate the growth of 'Hokke', *Pleurogrammus azonus*, on the basis of consideration of the seasonal change of mode in length frequency distribution. Data were collected from 1949 to 1956 in Hokkaido waters chiefly.

Results obtained here are summarized as follows:

1) It is estimated that the fish grow to about 21.5, 28 and 33 cm in body length in the first, second and third year of their life respectively.
2) The growth in the period from May to August may be quick, then extremely slow in the winter season.
3) At the young stage, the body length of the samples from the Japan Sea is greater than that of those from both the Sea of Okhotsk and the Pacific area but distinct differences are not recognized between each area in respect to the growth after the end of the first year of life.

筆者はホツケの体長資料を使用して成長の推定を試み，二三の知見を得たので報告する。

本文に入るに先だち，報告の御校閲を賜つた本学部淡水増殖学教室山本喜一郎教授，並びに研究を進めるに当つて幾多の御便宜をはかつて下さつた北水研大垣光平所長，佐藤栄資源部長，添田潤助函館支所長及び組織研究にたずさわつている北水研，北水試の各位，又資料の整理に尽力された当教室佐々木敏子嬢に感謝の意を表する。

材料及び方法

本研究に使用した体長資料は1949年6月から1956年6月までの間に蒐集したもので，その中体長約18cm以上のものは，周年操業される底曳網及び9月～12月の産卵期に於ける底建網，延縄並びに刺網で漁獲されたものである。[1] 体長18cm以下の資料（第1表）は，主として6月中旬～8月上旬に日高沿岸の定置網で漁獲されたもの及びたも網又は稚魚ネットにより12月～7月の期間に北部日本海並びにオホツク海で各々採集されたものである。

本報告では上記資料の月別又は採集日毎の体長分布に出現しているモードの季節的推移より成長を考察した。但し組成の不明な資料（第1表）では体長範囲の中央値を使用しているが，これ等の資料はいずれも成長の初期段階のものであり，又体長範囲も狭いからモードに代る値として取扱つている。

結果及び考察

全資料を日本海水域，オホツク海水域及び太平洋水域の三つに大別し，年次の区別をせずに体長モードの季節的変化を示したのが第1図で，20.5cm以上のものは月別資料のモードを又16.5cm以下のものは第1表

Table 1. Data on the larval and young stage

Sampling date	Region	Gear	Range of body length (cm)	Mode (cm)	Individual number
1949 Ⅵ.15-Ⅶ.28	Horoizumi	Set net	4.5-16.5	14.5	228
1950 Ⅶ.9	〃	〃	8.5-15.5	12.5	179
〃 Ⅶ.21	Kushiro	〃	9.5-11.5	10.5	21
1951 Ⅵ.26-Ⅷ.8	Horoizumi	〃	5.5-21.5	10.5 15.5	380
1952 Ⅵ.18	Kabuka	〃	14.5-18.5	16.5**	108
〃 Ⅵ.23-Ⅷ.5	Horoizumi	〃	6.5-21.5	11.5	12594
1953 Ⅴ.7	Kumaishi	〃	9.5-11.5	10.5**	3
〃 Ⅴ.7-12	Kamui point NW 10-40′ Ofuyu point NW 40-60′	Larval net	2.5-9.5	3.5	291
〃 Ⅵ.4	Oohana point	Set net	16.5		1
〃 Ⅵ.21	Mitsuishi	〃	8.5-13.5	11.5	50
〃 Ⅵ.16-Ⅶ.11	Horoizumi	〃	6.5-19.5	10.5 16.5	5396
1954 Ⅳ.2-3	North off Kamui point	Larval net	1.5-2.5	2.0	454
〃 Ⅵ.1-2	Notoro point N 30-40′	〃	2.3-6.8*	4.55**	40
〃 Ⅶ.12-24	Horoizumi	Set net	8.5-18.5	14.5	408
〃 Ⅻ.16-17	Off Takashima-Kamui point	Larval net	0.8-1.15*	0.98**	43
1955 Ⅰ.24-26	〃	〃	0.9-2.4*	1.65**	125
〃 Ⅱ.9	〃	〃	1.1-3.1*	2.1**	41
〃 Ⅲ.8-9	〃	〃	1.4-2.2*	1.8**	33
〃 Ⅴ.2	Aonai SSW-WSW 20-40′	Dip net		3.5	5
〃 Ⅴ.3	〃	〃	3.9-9.8	6.85**	5
〃 Ⅴ.21	Aonai NW 40′	〃	9.5-13.5	12.5	8
〃 Ⅴ.29	Mitare W 3/4 N 60′	〃	8.3-12.4	10.4**	3
〃 Ⅵ.4	Iwanai W/N 70.′5	Drift net	14.5-15.5	15.0**	3
〃 Ⅵ.12	Kamui point NW 100′	Dip net	8.5-9.5	9.0	6
〃 Ⅶ.23	Kushiro	Set net	11.5-14.5	12.5	30
〃 Ⅶ.17-26	Urupu Is. North 60-100′	Dip net	10.5-17.5	12.5	28
1956 Ⅴ.10-29	Aonai SSW-W 20-40′	〃	7.5-15.5	12.5	37
〃 Ⅵ.3-4	Aonai SSW-W 20-40′	〃	13.5-15.5	14.5	9

Data were collected by Hokkaido regional Fisheries Research Laboratory.
* Total length ** Median

の資料のモードを各々記入してある。これより距視的にみて三つのそれぞれ異なる推移を示す一連のモード群が伺われる。

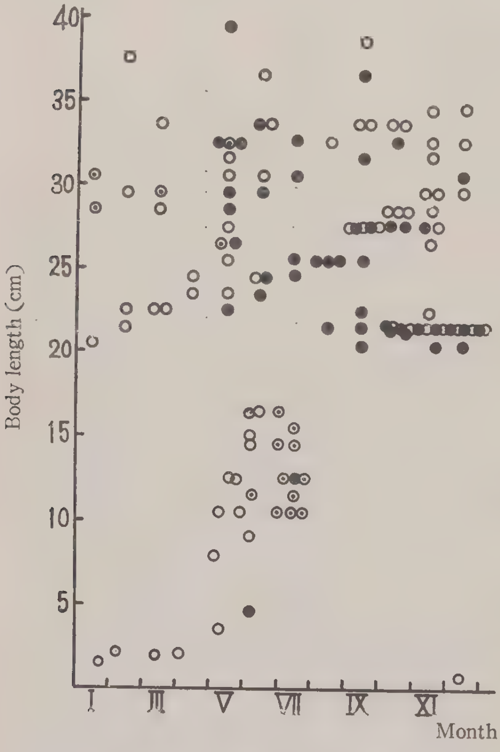


Fig. 1. Seasonal change in the mode of length distribution
○ Japan sea area ● Okkotsk sea area
⊙ Pacific area

第1は1月の1.65cmより始まるモード群で，3月～4月には1.8～2.0cmを示し，5月～6月と時間の経過と共にその値は急速に大きくなり，7月の最大値は16.5cmを示すようになる。これに続く18cm前後のものは7年間の資料に全くみられないが9～12月には21.5cm前後の処にこれに続くと思われるものがあらわれている。夏期に於ける急速なモードの移行は，こゝで用いられた幌泉の資料を年次別にし，更に資料採取日毎の体長組成に細分すればより明瞭となる（第2図）。即ち6月中旬のモードは10cm前後であるが以後8月上旬の15.5cmまで極めて明瞭な移行を示し，然もその移行は年により大きな差異は認められず，常に10cm前後に始まり2ケ月の間に15.5cmと約5cm移行している。又この一連のモード群とは別に17cm前後に別のモード群がみられるが出現の度合は少なく，これは1951年及び1953年の資料にだけ出現している。

第2群のモードの推移は，1月20.5cmに始まり12月の30cmに終る。その季節的な移行はほぼ直線的であるが，更に詳細にこれを検討するため北海道北西水域より得られた測定資料毎のモードの季節的変化を年次別に追求してみると（第3図），各年とも11月～3月の期間は20.5～22.5cmで比較的一定の値を示すが，以後4月から9月まではその値が急速に大きくなつている。1954年の資料では8月～9月の28cm前後に達したモードが11月～12月に再び安定した値を示している。

第3群のモードの推移は1月28.5～30.5cmから12月の32.5～34.5cmに至る移行で，モードの散らばりが大きいため第2群程明瞭な移行は認められない。

又，水域別にモードの出現状況をみれば（第1図），16.5cm以下の資料で日本海水域のものは12月～6月の間に約1cmから16.5cmに移行し，オホツク及び太平洋水域では資料が6月及び7月に限られていて，6月上旬に各々4.55及び11.5cmの各1資料がみられ，7月にはオホツク水域で12.5cmに，又太平洋水域では10.5～16.5cmの間にモードが出現している。従つて各水域の資料を十分比較することは出来ないが，モード値分布の傾向からみて日本海水域のものは他水域のものより体長が大きいと見做される。然しこれに続く10月～12月のモードでは日本海及びオホツク両水域の示す値は21.5cmではとんど差異は認められない。第2群のモードの推移についてみれば，オホツクのものが日本海水域のものより幾分小さい値を示しているが判然とした差異は認められず，又第3群のモードでは各水域とも散らばりが大きく，差異を論ずることは出来ない。

以上の結果よりホッケの成長を考察しよう。

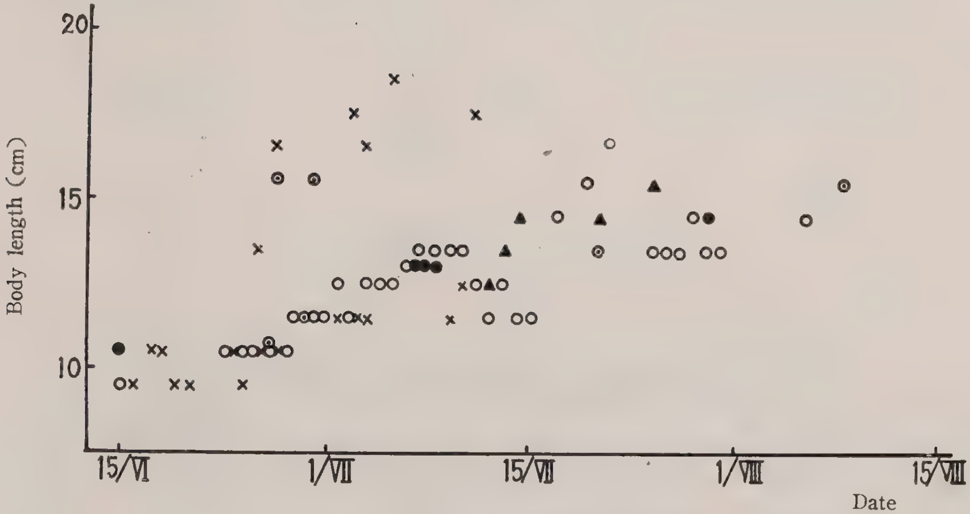


Fig. 2. Daily change in the mode of length distribution from Horoizumi
● 1949　⊙ 1951　○ 1952　× 1953　▲ 1954

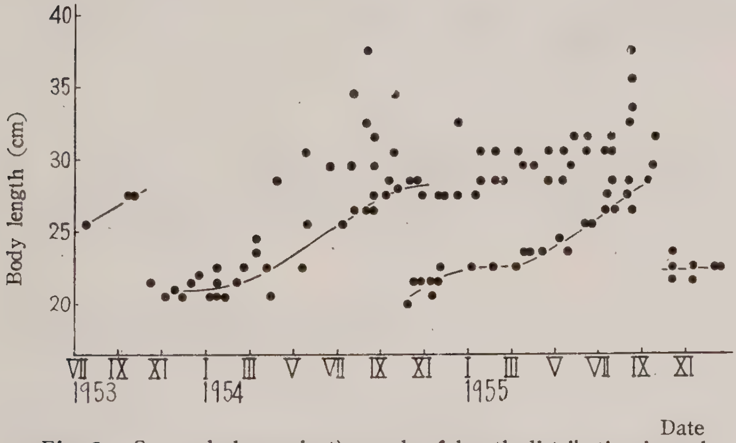


Fig. 3. Seasonal change in the mode of length distribution in each sample from the north-western area of Hokkaido

ホッケの産卵期は９月から12月の間で，北海道南部日本海沿岸，利尻・礼文及び知床の主産卵場で時期的に幾分異なる。卵は室内発生実験[2]によれば，水温条件10°Cで孵化まで約65日を要し，孵化時の体長は0.9 cm 前後である。各産卵場の海況条件は調査されていないが，北海道南部沿岸の表面水温* の状況をみれば（第４図），10月～１月の期間で約21～6 °Cの範囲にある。従つて12月～４月に稚魚ネットで採集された体長モード 1.0～4.5cm のものは明らかに前産卵期に産卵された卵から孵化した稚魚群で，その急速なモード値の上昇は幼魚期の速な成長を示すものと考えられ，その年の12月には体長21.5cm前後となる。又幌泉の日別体長組成（第２図）に現れた 17cm 前後のモード群は，初年の成長が約21.5cmであり，後述の如く第２年目の６月～７月には体長 24～25cm に成長するという推定にもとづけば，６月中旬～８月上旬の間に体長約 10 cm から 15cm に成長する魚群と年齢を異にするものではなく，孵化年次は同じであるが異つた産卵場で孵化

* 北水研資料

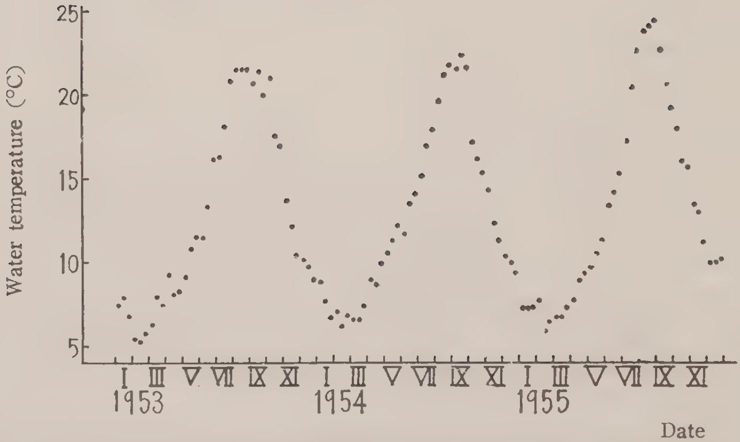


Fig. 4. The surface temperature (°C) in the coastal waters of southern Hokkaido

した魚群がその年によつて混入して来るものと考えられる。

又上記の第2，第3のモード群はそれぞれ孵化後2年及び3年目の魚群をあらわすものと判断され，従つてそれ等の成長は2年目で約20.5cmから30cm，3年目で28.5〜30.5cmから32.5〜34.5cmとなり，産卵後満2年及び3年ではゞ28及び33cm前後の体長に達するものと推定される。又季節的には5月〜8月に成長が著るしくよく，11月〜3月の冬期間は遅滞期と考えられる。3年以上の成長については資料が散漫なため不明である。

水域による成長を比較すれば，孵化後数ケ月間に採取された資料で日本海水域のものはオホツク及び太平洋両水域のものより体長が大きい。これは各幼魚群の由来する産卵場における孵化時期の相異によるものであろうが，その年の秋には日本海及びオホツクのいずれの水域でも体長21.5cm前後に達し，以後2年目迄の成長に論ずる程の差異は認められない。又3年目の水域による成長の差異については本資料より推論できない。

以上，体長資料より大略の成長を考察したが，雌雄別及び高齢魚の成長については他の調査にまたねばならない。

摘　　要

筆者は1949年より1956年までの体長資料を使用し，体長分布に現れているモードの季節的推移よりホッケの成長を考察した。その結果は次のように要約される。

1) 産卵後1，2年及び3年で各々21.5，28及び33cm前後の体長に成長するものと推定される。
2) 5月〜8月に成長は著るしく，11月〜3月の冬期間はその遅滞期と考えられる。
3) 孵化後数ケ月間に採集された資料では，日本海水域のものがオホツク及び太平洋両水域のものより体長が大きい。然し以後2年目迄の成長に水域による差異は認められない。

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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

IX. Method for Preventing the Formation of the Crystals during the Processing of the Cans

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I. Formation of the crystals in canned foods to which gelatine, agar or S.L.D. were added in order to raise the viscosity of the juice

In the previous paper, I of this series[1], the authors have suggested a relationship between the viscosity of the juice of canned foods and formation of the crystals. Also in paper VI[2], observations were reported on the formation of the crystals in the solution of chemical components of the crystal to which gelatine or agar was added in order to raise the viscosity. According to the observations, the higher the viscosity of the juice is, the slower the crystallization becomes, but over a long period the crystals will surely be formed; when the crystals once begin to form, they are likely to grow to large ones having regular surfaces.

The authors have here observed the formation of the crystals in canned crab or canned salmon to which gelatine, agar or S. L. D. were added in order to raise the viscosity of the juice. S. L. D. is the commercial name of a powder of the hydrolyzate of starch, made by Mimatsu Co. Ltd.

1. In the case of canned crab

(*1*) *Sample and experimental method*

When the canned crab was prepared from the raw material caught off Nemuro, the meat was packed with gelatine or agar of 0.45~0.075% of the total quantity of the content. The cooling of the cans was done in a water tank. The sample cans were brought to the laboratory and opened after 50 days; the formation of the crystals in the cans was observed. Experiment was carried out following the same methods as were described in paper VIII[3].

(*2*) *Experimental results*

Results obtained are shown in Tables 1 and 2.

As seen in these two tables, the higher the specific viscosity became as a result of the addition of gelatine, the smaller the size became of the crystals formed in the cans. When the specific viscosity was above or below a boundary of 2.4, the crystals formed were small or large respectively. In the case of the addition of agar, the specific

Table 1. Formation of the crystals in canned crab to which gelatine was added

Concentration of gelatine (%)			0.45	0.38	0.30	0.23	0.15
Color of meat			Good	Good	Good	Good	Good
Taste of meat			Good	Good	Good	Good	Good
State of juice		Viscosity (η/η_0)	2.7	2.6	2.4	2.3	2.1
		V.B.-N (*mg*%)	26.1	23.5	24.1	22.8	9.4
		Tot.-N. (%)	1.11	1.13	1.16	1.10	1.21
		Soluble Mg (*mg*%)	1.6	1.8	1.2	0.5	0.2
Crystal in juice	Regular crystal	Number (per *cc*)	56×10^4	56×10^4	12×10^4	8×10^4	8×10^4
		Size (μ)	25×5	26×9	21×6	80×38	38×2
	Amorphous	Number (per *cc*)	80×10^4	100×10^4	56×10^4	80×10^4	80×10^4
		Size (μ)	12×3	13×3	13×3	6×3	6×3
Size of crystals on parchment paper		Top (μ)	250×60	250×65	3×3	1000×2000	660×170
		Side wall (μ)	200×45	250×65	2×2	1000×2000	660×160
		Bottom (μ)	250×65	250×65	40×10	1000×2000	660×170

Table 2. Formation of the crystals in canned crab to which agar was added

Concentration of agar (%)			0.30	0.24	0.15	0.075
Color of meat			No good	No good jelly state	No good jelly state	No good
Taste of meat			Good	Good	Good	Good
State of juice		Viscosity (η/η_0)	10	5.2	3.3	2.3
		V.B.-N. (*mg*%)	34.2	33.9	32.2	39.0
		Tot.-N. (%)	1.08	0.93	0.10	——
		Soluble Mg (*mg*%)	5.2	4.5	3.0	3.4
Crystal in juice	Regular crystal	Number (per *cc*)	8×10^4	——	8×10^4	20×10^4
		Size (μ)	9×6	——	38×12	70×12
	Amorphous	Number (per *cc*)	80×10^4	80×10^4	22×10^4	80×10^4
		Size (μ)	25×25	25×25	25×25	12×12
Size of crystals on parchment paper		Top (μ)	——	——	——	——
		Side wall (μ)	190×60	190×60	430×200	120×250
		Bottom (μ)	600×250	250×100	820×220	120×250

viscosity increased considerably. When the specific viscosity became over 5.2, numerous and irregular crystals tended to form in the cans, but some difference was observed only in size of the crystals formed. In such cans, the content is jellified and has poor merchandise value.

According to the results obtained, a relation between state of the crystallization and the amount of added materials was observed respectively to be different in each of the cans to which gelatine or agar was added. Size of the formed crystals became small in accordance with the increasing of the specific viscosity or the amount of added gelatine. In the cans to which were added various amounts of agar, difference in the size of formed crystals was not observed, but difference in shape was observed. The number of irregular crystals increased with larger amount of added agar. However, in the juice the soluble magnesium which has not reacted with other components of the crystal remained in large quantity in those cans to which the larger amount of gelatine or agar was added. This happens, because the supersaturated solution is stabilized with the added high polymer; then, the crystal will grow over a long time with probability of growing to a large size. However, the growing velocity of the formed crystals will be reduced considerably because of the larger number of crystals formed as a result of the addition of the larger amount of these substances.

2. In the case of canned salmon

(*1*) *Sample and experimental method*

When the canned salmon has been prepared as customarily from the raw materials (*keta* salmon) caught in the northern sea (Hokuyō), the meat was packed with gelatine of 0.48~1.88% or S. L. D. of 0.04~0.80% of the total quantity of the content. The cooling of the cans was done as usual. The cans prepared by the method described above were employed for experiment as the sample, and opened after one and a half years; the formation of the crystals in the cans was observed in the same manner as that described in paper VIII[3].

(*2*) *Experimental results*

Results obtained are shown in Tables 3 and 4.

As seen in Tables 3 and 4, the larger the amount of the added gelatine or S. L. D. was the less became the number of large crystals formed in the cans. When the added amount of gelatine was over 3.5 *g* per a can (1.4%), visible crystals of over 1 *mm* size were not formed. In such can, the content is jellified and has poor merchandise value.

In the cans to which S. L. D. was added, the pH values declined toward the acidic side owing to the larger amount of added S. L. D. When the added amount of S. L. D. was over 1 *g* per a can (0.4 %), the formation of large crystals were not observed. In

Table 3. Formation of the crystals in canned salmon to which various amount of gelatine was added

Sample No.	Added amount of gelatine (g/can)	Color of meat	Viscosity (η/η_0)	Crystals on meat: Visible crystals		Crystals in juice: Visible crystals		Crystals in juice: Microcrystals	
				Size (μ)	Number (/can)	Size (μ)	Number (/can)	Size (μ)	Number (/cc)
1	0	Good	2.9	1000～3500×800	30	500～3000×200～1000	20	32～98×16	7×10⁴
2	1.2	Rred brown	4.4	2000×1000	2	1000～2000×200～1000	8	50～160×16～32	30×10⁴
3	2.4	Yellow brown	6.1	3000～3500×800～1000	6	3000～3500×800～1000	3	65～190×16～32	17×10⁴
4	3.5	Brown jelly-state	7.0	——	None	——	None	32～110×16	30×10⁴
5	4.7	Brown jelly-state	7.7	——	None	——	None	32～65×16	3×10⁴

Table 4. Formation of the crystals in canned salmon to which various amount of S.L.D. was added

Sample No.	Added amount of S.L.D. (g/can)	Color of meat	pH	Viscosity (η/η_0)	Crystals on meat: Visible crystals		Crystals in juice: Visible crystals		Crystals in juice: Microcrystals	
					Size (μ)	Number (/can)	Size (μ)	Number (/can)	Size (μ)	Number (/cc)
1	0	Good	6.2	2.9	2000～5000×200～1000	10	3000～6000×800～1000	9	32～64×16	3×10⁴
2	0.1	Good	6.0	3.5	2000～7000×1000	92	650～820×160～250	2	——	None
3	0.2	Good	5.9	3.8	1000～5500×200～1000	90	1500～3100×160～650	8	——	None
4	0.5	Good	5.7	3.7	1000～4000×200～900	58	820～1200×320～570	6	——	None
5	1.0	Good	5.7	4.3	——	None	——	None	——	None
6	2.0	Good	5.1	4.6	——	None	——	None	——	None

such cans, the reduction of the formation of crystals will be considered to be caused not only by the increased specific viscosity, but also by the decreasing of pH value. As seen in the results described above, if comparatively larger amount of agar, gelatine or S. L. D. were added in order to rise the specific viscosity of the juice in the cans, the formation of the larger crystals may be prevented. But, in such cans, the content will have poor merchandise value in appearance.

II. The addition of complex ion-making agents as a method for preventing the formation of the crystals

In order to prevent the formation of scales which are an accumulation of Ca or Mg

salts in a boiler from boiler water, antiscales have been used. The practice is based on the principle that antiscales unite with Ca or Mg of divalent metallic ion to make soluble complex salt and thus prevent the precipitation of Ca or Mg salts. In the U. S. A., to prevent the formation, in canned sea foods, of glass-like of struvite, which consists of Mg, NH_4 and PO_4 ions, certain antiscales, *e. g.* sodium hexametaphosphate have been used. However, the use of antiscales in canned foods must be examined, because such foods with added antiscales have unfavourable taste or comparatively worse quality from the health point of view.

Sodium hexametaphosphate combines with Mg in the canned foods as a soluble magnesium complex salt according to the following formula.

$$[Na_{2n}(PO_3)_{3n}] + nMg \rightarrow Na[Mg_n(PO_3)_{3n}]$$

This soluble magnesium complex does not precipitate in the can. The authors have used sodium hexametaphosphate for preventing the formation of $MgNH_4PO_4 \cdot 6H_2O$ crystals in the canned crab.

1. Test tube experiments on the influence of the addition of sodium hexametaphosphate upon the formation of the crystals

In order to determine the adequate dose of sodium hexametaphosphate, solutions of various concentrations of the reagent were respectively added to the mixed solution of the chemical constituents of $MgNH_4PO_4$, magnesia mixture and sodium phosphate solutions, and the formation of the crystals was observed.

(1) *Experimental method*

Each 0.01 Mol solution of magnesia mixture and sodium phosphate solutions was instantly mixed in separate test tubes, to which various concentrations of 1～0.1% of sodium hexametaphosphate were added. Then the formation of the crystals was observed.

(2) *Experimental results*

Results obtained are shown in Table 5.

As seen in Table 5, when the chemical constituents were instantly mixed after the addition of sodium hexametaphosphate, the crystals having regular surface did not appear even after 24 hours, and the size of the crystals which were formed was smaller than that in material to which no agent had been added. After long storing of the test tubes to which a small quantity of the complex ion-making agent (below 0.5 %) had been added, the crystals came to have regular surface. This is perhaps owing to the formation of Mg-complex and to the increase in the viscosity of the solution. This was also suggested from the results reported in the previous paper, VI[2]. However those crystals formed were microscopic and invisible. The amount of Mg in the mixed solution of magnesia mixture and sodium phosphate was 0.0122 % and the amount of Mg in the

Table 5. States of the formation of the crystals in the solution to which various amount of sodium hexametaphosphate were added

Concentration of sodium hexametaphosphate (%)		0	1	0.5	0.25	0.1
Leaving time 5 (min.)	Form	C	E	E	E	E
	Size (μ)	380×19	1×1.5	1×1	1×1	3×2
24 (hrs.)	Form	C	E	E	E	E, C
	Size (μ)	540×50	No change	No change	1.5×2	6×2
48 (hrs.)	Form	C	E	C, E	C	C
	Size (μ)	No change	No change	——	10×2	6.5×2

Note: Singns which are described in this table show the states of the formed crystals as follow. C=columnar crystal, E=amorphous

juice of canned crab is 0.008%[1]; the amount of Mg in the mixed solution agrees with the amount of Mg in the juice of canned crab. In order to form only microscopic crystals, additon of 0.5~1.0% of sodium hexametaphosphate is considered to be adequate.

2. The influence of the addition of sodium hexametaphosphate upon the formation of the crystal in canned foods

1. In the case of canned crab

(1) *Sample*

When the cans were prepared in customary manner from the raw material caught off Nemuro, the meat was packed with sodium hexametaphosphate (Mascolin S, made by Taiheiyō Kagakukogyō Co. Ltd.) or tablet of sodium hexametaphosphate made by Scientific Salting Co. in the U. S. A. The added amount of the complex ion-making agent is shown in Table 6.

The cans containing various of the reagent were processed as customary and opened after 50 days.

(2) *Experimental method*

By the method described in a previous paper[3], determinations of the qualities of the contents, specific viscosity, amounts of volatile basic nitrogen, total nitrogen and the soluble magnesium in the juice were carried out. Also, the shape, number and size of the crystals formed in those cans were observed microscopically.

(3) *Experimental results*

Results obtained are shown in Tables 7 and 8.

Table 6. Added amounts of sodium hexametaphosphate for canned crab

Sample	Added amount of tablet of sodium hexametaphosphate (made in U.S.A.) (g/can)	Added amount of sodium hexametaphosphate (g/can)	Added rate of sodium hexametaphosphate for the amount of the contents (%)
1	1.5	0.45	0.23
2	0.90	0.30	0.15
3	0.69	0.23	0.11
4	0.45	0.15	0.075
5	0.27	0.09	0.045
6	0.09	0.03	0.015
Sample	**Mascolin "S"**	**Added amount of sodium hexametaphosphate**	**Added rate of sodium hexametaphosphate for the amount of the contents**
1	0.45	0.45	0.23
2	0.30	0.30	0.15
3	0.23	0.23	0.11
4	0.15	0.15	0.075
5	0.09	0.09	0.045
6	0.03	0.03	0.015

Table 7. States of the formation of the crystals in canned crab to which various amounts of sodium hexametaphosphate tablets were added

Concentration of sodium hexametaphosphate (%)		0.23	0.15	0.11	0.075	0.045	0.015
States of meat	Color	White pale yellow	White pale yellow	Good	Good	Good	Good
	Taste	Bitter	Bitter	Good	Good	Good	Good
Viscosity (η/η_0)		3.0	2.5	2.4	2.5	2.5	2.3
V. B-N. (mg%)		29.7	32.8	——	35.4	34.3	24.7
Total-N (%)		0.999	1.12	1.06	1.09	0.453	1.03
Mg (%)		0.0018	0.0049	0.0054	0.0037	0.0033	0.0054
Crystals in juice	Number (/cc)	80×10^4	80×10^4	80×10^4	8×10^4	24×10^4	40×10^4
	Size (μ)	3.8×3.8	3.8×3.8	13×9	10×12	31×5	45×13
	Form	E	E	E, C	C	C	C
Crystals on parchment paper	Top	—	—	—	—	—	+
	Side wall	—	—	—	—	+	+
	Bottom	—	—	+	+	+	+

Note: Singns which are described in this table show the states of the formed crystals as follow. + = crystals of above 150μ, − = no crystal, C=columnar crystal, E=amorphous

Table 8. States of the formation of the crystals in canned crab to which various amounts of sodium hexametaphosphate made by Taiheiyō Kagaku Co. were added

Concentration of sodium hexametaphosphate (%)		0.23	0.15	0.11	0.075	0.045	0.015
States of meat	Color	Yellow	Yellow	Yellow	Good	Good	Good
	Taste	Bitter	Bitter	Sour	Good	Good	Good
Viscosity (η/η_0)		1.9	2.1	1.9	2.1	2.0	2.5
V. B-N. (*mg%*)		31.5	38.2	35.4	36.2	33.9	31.5
Total-N (%)		0.984	1.011	1.02	1.07	1.04	1.09
Mg (%)		0.0081	0.0023	0.0029	0.0023	0.0088	0.0045
Crystals in juice	Number (/*cc*)	100×10^4	——	22×10^4	50×10^4	22×10^4	22×10^4
	Size (μ)	4×4	4×4	38×25	25×13	57×19	110～210×20
	Form	E	E	C	C	C	C
Crystals on parchment paper	Top	—	—	—	—	−, +	+
	Side wall	—	—	—	—	+	+
	Bottom	—	—	—	−, +	+	+

Note: Singns which are described in this table show the states of the formed crystals as follow. + = crystals of above 150μ, − = no crystal, C = columnar crystal, E = amorphous

As seen in Tables 7 and 8, in the cans to which sodium hexametaphosphate was added in various concentrations, the formation of the crystals was varied with the added amounts of the reagent. When the added amount was 0.3 g per one can and was above 0.15 % of the amount of the contents, the crystals formed in the cans were irregular shaped, large in number and 3～8 μ in size. If the amount of sodium hexametaphosphate was less than 0.15 *g* (0.075 % of the content), crystals generated having a more regular surface and larger size. No intimate relation was observed among the added amount of sodium hexametaphosphate and specific viscosity or the amounts of volatile basic nitrogen, total nitrogen and soluble magnesium. But, remarkable relation was observed between the commercial qualities of the content of the cans and the added amounts of the reagent. The larger the added amount of the reagent, the worse were the color and the taste of the contents. When the amount of the reagent added to the cans was over 0.15 %, the contents of the canned food became pale yellow in color and bitter in taste. In order to prevent the formation of the crystal in the canned crab, the amount of sodium hexametaphosphate added per a can must be limited commercially below 0.11 % of that content (0.23 *g*. per one can).

2. In the case of the canned salmon

(*1*) *Sample*

On the floating cannery, when the cans were processed in customary manner from the

keta salmon caught at the northern sea (Hokuyō), the meat was packed with tablets of sodium hexametaphosphate, Mascolin "M" ($M_n + 2P_nO_{3n+1}$) and Mascolin "T" ($M_n + 2P_nO_{3n+1}$) which are kinds of sodium hexametaphosphate. The amounts of the complex ion-making agent are shown in Table 9.

Table 9. Added amounts of sodium hexametaphosphate for canned salmon

Sample	Added amount of tablet of sodium hexametaphosphate (made in U. S. A.)	Added rate of sodium hexametaphosphate for the amount of the contents
P 1	0 *g/can*	0 %
P 2	0.7	0.28
P 3	1.18	0.47
P 4	1.88	0.75
P 5	2.35	0.94
P 6	3.53	1.41
Sample	**Added amount of Mascolin "M"**	**Added rate of Mascolin "M" for the amount of the content**
M 1	0.24 *g/can*	0.096 %
M 2	0.70	0.28
M 3	1.18	0.47
M 4	2.35	0.94
M 5	3.53	1.41
M 6	4.70	1.88
M 7	7.05	2.82
Sample	**Added amount of Mascolin "T"**	**Added rate of Mascolin "T" for the amount of the content**
T 1	0.24 *g/can*	0.096 %
T 2	0.70	0.28
T 3	1.18	0.47
T 4	2.35	0.94
T 5	3.53	1.41
T 6	4.70	1.88
T 7	7.05	2.82

The cans containing various amounts of the reagents were seamed, cooled as customarily after sterilization, and opened after one year.

(2) *Experimental method*

The state of the formed crystals in the cans to which the reagents were added was observed by the same method described in the previous paper[3].

(3) *Experimental results*

Results obtained are shown in Tables 10, 11 and 12.

As seen in these tables, the state of the formation of the crystals in the cans to which the complex ion-making agents were added varied with the added amounts of the reagent.

Table 10. States of the formation of the crystals in canned salmon to which various amounts of tablets of sodium hexametaphosphate were added

Sample No.	Added amount of tablet	pH	State of juice	States of crystals in juice						Crystals on meat	
				Amorphous		Microcrystal		Macrocrystal		Macrocrystal	
				Size (μ)	Number	Size (μ)	Number (/cc)	Size (μ)	Number (/can)	Size (μ)	Number (/can)
P 1	0.0	6.2	Good	24×16	Many	16∼48×16	5×10^1	240∼3300×32∼510	52	1500∼5500×1000∼1500	7
P 2	0.7	6.4	Good	16×16	Many	48×16	2×10^4	1000∼5000×500∼2000	26	1000∼3000×500∼1500	24
P 2	0.7	6.4	Good	16×16	Many	32×16	2×10^4	1000∼12000×500	20	1000∼1200×500∼800	18
P 3	1.18	6.4	Good	16∼32×16	Many	48×16	1×10^4	1000∼2500×200∼1000	15	——	None
P 3	1.18	6.4	Good	32×16	Many	32×16	1×10^4	1000∼1150×200∼500	14	500∼1200×200∼800	3
P 4	1.88	6.5	Good	16×16	Many	16× 8	1×10^4		None		None
P 4	1.88	6.5	Good	16× 8	Many	32×16	1×10^4		None		None
P 5	2.35	6.5	Muddy	16×16	Many	——	None		None		None
P 5	2.35	6.5	Muddy	16×16	Many	——	None		None		None
P 6	3.53	—	Muddy	16×16	Many	16× 8	1×10^4		None		None
P 6	3.53	—	Muddy	16×16	Many	——	None		None		None

Table 11. States of the formation of the crystals in canned salmon to which various amount of Mascolin "M" were added

Sample No.	Added amount of Mascolin "M" (g/can)	pH	State of juice	State of meat	Crystals on meat		States of crystals in juice			
					Visible crystal		Microcrystal		Visible crystal	
					Size (μ)	Number (/can)	Size (μ)	Number (/cc)	Size (μ)	Number (/can)
M 1	0.24	6.0	Good	Good	——	None	32∼160×16∼32	2×10^4	——	None
M 2	0.70	5.4	Good	Pale brown	——	None	32∼150×16∼32	2×10^1	——	None
M 3	1.18	6.4	Good	Pale brown	——	None	32∼100×16∼32	1×10^4	——	None
M 4	2.35	6.0	Muddy	Pale brown	——	None	32×16	1×10^4	——	None
M 5	3.53	6.0	Muddy	Pale brown	——	None	——	None	——	None
M 6	4.70	5.8	Muddy	Pale brown	——	None	——	None	——	None
M 7	7.05	5.3	Muddy	Pale brown	——	None	——	None	——	None

Table 12. States of the formation of the crystals in canned salmon to which various amount of Mascolin "T" were added

Sample No.	Added amount of Mascolin "T" (g/can)	pH	State of meat	State of juice	Crystals on meat: Visible crystal Size (μ)	Crystals on meat: Visible crystal Number (/can)	States of crystals in juice: Microcrystal Size (μ)	States of crystals in juice: Microcrystal Number (/cc)	States of crystals in juice: Visible crystal Size (μ)	States of crystals in juice: Visible crystal Number (/can)
T 1	0.24	6.0	Good	Good (yellow)	—	None	32~65×16~32	3×10^4	—	None
T 2	0.70	5.6	Discolor	Good (yellow)	—	None	32~65×16~32	5×10^4	—	None
T 3	1.18	6.3	Discolor	Muddy brown	—	None	32~65×16~32	1×10^4	—	None
T 4	2.35	6.4	Discolor	Muddy brown	—	None	32×16	1×10^4	—	None
T 5	3.53	6.3	Discolor	Muddy brown	—	None	—	None	—	None
T 6	4.70	6.3	Discolor	Muddy brown	—	None	—	None	—	None
T 7	7.05	5.7	Discolor	Muddy brown	—	None	—	None	—	None

When the tablet of sodium hexametaphosphate was added in quantity over 0.94 % of the content of the can (2.35 *g* per one can), neither the microcrystals, nor macrocrystals (visible) were formed.

In the cans to which the reagent of 1.88 *g* (0.75 % of the content in the can) was added, a few microcrystals were formed, but no visible crystal generated which would be commercial obstacles. However, when the added amount of the reagent was below 0.75 % of the content of the can, the formed crystals were not only microcrystals, but also macrocrystals. In the cans to which various amounts of the reagents were added, the relation between the qualities of the juice of the can and the added amounts of the reagent was observed.

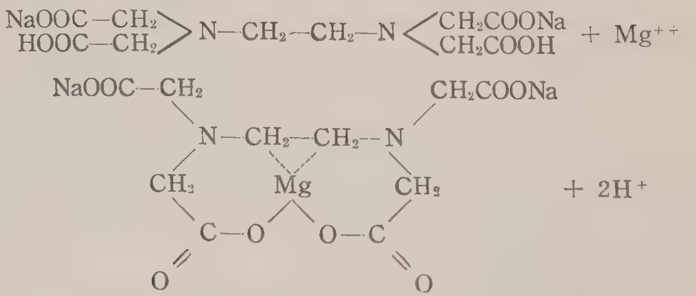
When the added amount of reagent was over 0.94 % (2.35 *g* per one can), the juice of the can became muddy and was whitish turbid. On the contrary, when the added amount of the reagent was below 0.75 % (1.88 *g* per one can), no difference between the cans and blank one was observed in the quality of the juice. In the manufacture of canned salmon, in order to prevent the formation of crystals without loss of commercial merchandise value, the added amount of the tablet of sodium hexametaphosphate should be 0.75 % of the content of the can (1.88 *g* per one can).

As seen in Tables 11 and 12, in the cans to which the added amount of Mascolin "M" or "T" was over 0.09 % of the content of the can (over 0.24 *g* per can), visible crystals were not formed. In the cans to which the added amount of Mascolin "M" or "T" was above or below 0.94 % as a boundary for the content of the can, visible crystals or microcrystals were not formed in the former, but microcrystals were formed in the later respectively. In such cans, the pH values declined toward the acidic side dependent upon the larger amount of Mascolin "M" or

"T" added. When Mascolin "M" or "T" was added to the cans, the state of the content of those cans differed. In the cans to which Mascolin "M" was added, the juice was pale yellow and transparent. In the cans to which Mascolin "T" was added, juice of the canned food was brown and muddy. Also, remarkable differences in the color or appearance of the meat content of the cans were observed. It is interesting that in the cans to which Mascolin "M" was added, the larger the added amount of the reagent, the stronger was the elasticity of the meat in the cans, also the color of the meat became brown. In the cans to which Mascolin "T" was added, the elasticity of the meat was not changed in proportion to the added amounts of the reagent, but the color of meat became pale, and the meat was discolored. According to these results, it is evident that Mascolin "M" or "T" is available to prevent the formation of the crystals, and the amount of the reagents is considered to be adequate at 0.24 ~ 0.7 *g* per can (0.096 % ~ 0.28 % of the content of the can).

III. The influence of the addition of E.D.T.A. (Ethylendiamine tetraacetate) upon the formation of the crystals

Recently, in the field of inorganic analysis, E. D. T. A. has been generally employed in accordance with the chemical action in which the disodium salt (disodium dihydrogen ethylen diamine tetraacetate dihydrate) form quantitatively a stable complex salt with calcium ion (Ca^{++}) or magnesium ion (Mg^{++}) as described in the following.



Here, the authors will report the observations on the influence of the addition of E. D. T. A. upon the formation of the crystals, the quality of the content of canned foods and the empty cans.

1. The influence of the sheet iron of an empty can upon the reactivity of E. D. T. A. reagent

(*1*) *Experimental method*

One-fiftieth N E. D. T. A. solution and N/50 magnesium sulfate solution were prepared. Twenty *cc* of N/50 E. D. T. A. solution was diluted to 250 *cc*. In order to know the reactivity of the diluted E. D. T. A., 10 *cc* of the solution was titrated with

N/50 magnesium sulfate solution. The remaining volume, 240 *cc*, of the diluted E. D. T. A. solution was poured into an empty can (half pound, flat). This can was seamed, heated at 5 *lbs* for 85 minutes and then was cooled in running water. The can was left for 40 days. The change of the reactivities of the processed E. D. T. A. solution was estimated by titrating with N/50 magnesium sulfate solution.

(2) *Experimental results*

Results obtained are shown in Table 13.

Table 13. The change of the reactivities of the processed E.D.T.A. solution

Reactivity of non-processed solution	2.31 *mg* Mg/100 *cc* E. D. T. A.
Reactivity of processed solution after 40 days	0.91 *mg* Mg/100 *cc* E. D. T. A.
Reducing rate of reactivity of processed solution (%)	60.5

Table 14. Added amount of E. D. T. A.

Sample No.	Amount of crab meat	Amount of juice	Added amount of N/50 E.D.T.A. solution	Added amount of dist. water
1	5 *g*	0 *cc*	5 *cc*	0 *cc*
2	5	0	10	0
3	5	0	15	0
4	5	0	20	0
5	5	0	0	10
6	5	2	10	0

As seen in Table 13, the reactivity of E. D. T. A. solution which was poured in the can and was processed as in usual canning declined to 39.5 % in comparision with the reactivity of the non-processed solution.

2. The influence of E. D. T. A. upon the formation of the crystals in test tubes

(1) *Sample and experimental method*

In a can of crab (*Erimacrus isenbeckii*) which was prepared in the author's laboratory, the ratio of meat and juice was determined to be 5:2. Here, as shown in Table 14, each 5 *g* of the meat taken from that can was placed in six empty test tubes, and 2 *cc* of the juice separated was poured into one of the test tubes. Various amount of N/50 E. D. T. A. solution were poured into all the test tubes.

All those test tubes were closed with cotton stopper and heated at 5 *lbs* for 85 minutes as usual, cooled and then left for 40 days. After the leaving, the changes in

the color of the contents in the test tubes were observed. The juice separated from those test tubes was diluted with dist. water to 50 *cc*. Several drops of 1 % Na_2S solution was added to 10 *cc* of the dilute in order to counteract the obstacles to complex-making with $Fe^{++}$and Cu^{++}. Then 1 *cc* of the buffer solution was added to make pH 10.0, accompanied with indicator solution (alcoholic solution of eryochrome black "T"). The remaining amount of Mg^{++} which did not form E. D. T. A.- complex was estimated with N/50 E. D. T. A. solution and the amount of E. D. T. A. was beforehand back titrated with N/50 $MgSO_4$.

(2) *Experimental results*

Results obtained are shown in Table 15.

Table 15. Amount of E. D. T. A. required to prevent the formation of crystals in canned crab

Sample No.	1	2	3	4	5	6
Color of meat	No-change	No-change	No-change	No-change	No-change	No-change
Presence of free Mg ion which did not form complex-salt	+	+	−	−	+	+
Amount of E.D.T.A. required to the complex-salt	18.8 (*mg*)	24.3	26.1	37.2	15.6	32.4
Amount of E.D.T.A. required per one can of canned crab	0.34 (%)	0.43	0.47	0.67	0.28	0.51

As seen in Table 15, the dose of E. D. T. A. to be used for canned crab is clarified to be 0.34~0.67 % of the amount of the content of the can. In No. 5 sample can in which distilled water was added in place of E. D. T. A. solution, the amount of magnesium which dissolved into the juice in the can was slight. This is owing to the formation of complex salt from E. D. T. A. and magnesium or calcium in other cans.

No change in the color of the meat resulting from the addition of E. D. T. A. was observed. Therefore, E. D. T. A. has a preventive effect for the formation of struvite in the canned foods.

However, as a decrease in the reactivity of E. D. T. A. was observed from the result of Experiment 1, the using dose of E. D. T. A. is 0.85~1.7 % per can (net content: 200 *g*) of canned crab, considering from the loss of 60 % of the reactivity. This dose must be tried practically to prevent the formation of the struvite in the canned foods. But here the approximate using dose has been determined.

Summary

The authors have observed the formation of the crystals in canned crab or canned salmon to which gelatine, agar, or S. L. D. was added in order to raise the specific

viscosity. Thus it was clearly demonstrated that the higher the specific viscosity in the juice of the cans became, the less the number of the formed crystals became. However, when the agar, gelatine or S. L. D. needed for preventing the formation of the larger crystals was added, the contents had poor merchandise value. The amounts of agar, gelatine or S. L. D. needed for preventing the crystal formation were determined.

The formation of the crystals has been said to be inevitable under the condition of the existence of the components of the crystals, because the solubility of $MgNH_4PO_4 \cdot 6H_2O$ crystal was rather small[4]. In previous paper[3], the authors have investigated the mechanism of the formation of the crystals and made it clear that the size of the formed crystals may be rendered as small as possible by rapid cooling of the can after heating. However, in the use of such a method, it is practically impossible completely to prevent the formation of the crystals. For the purpose of the prevention of the formation of the crystals, it will be preferable that some one sort of the components of the crystals exchange into a soluble compound. Here, in order to prevent the formation of the crystals owing to the changing of the magnesium of the compnents into the soluble complex salt, various amounts of sodium hexametaphosphate, Mascolin "M", Mascolin "T" or E. D. T. A. were added to the canned crab or the canned salmon, and the states of the formation of the crystals were observed. Then the added amount of various complex ion-making reagents which were needed for the complete prevention of the formation of the crystals were determined. According to the results obtained, in the canned crab or canned salmon, to which 0.11~0.22 % of sodium hexametaphosphate, when 0.1~0.28 % of Mascolin "M" or Mascolin "T", and 0.34~0.67 % of E. D. T. A. were added, the formation of the crystals was prevented. The influence of the complex ion-making agents upon the commercial qualities of the cans was varied with the added amount. In the canned crab, when the added amount of sodium hexametaphosphate was over 0.11 %, the meat became pale yellow in color, and bitter to the taste. In the above described canned crab after it was opened a white substance precipitated in the can, and the merchandise value declined. In the canned salmon, when the added amount of Mascolin "M" was over 0.28 %, the meat was scarcely changed in taste, but a part of the meat became pale brown in color. In such cans, the meat was elastic and the phenomenon of meat crumbling did not occur. In the canned salmon to which Mascolin "T" was added, the meat became discolored and pale. When the added amount of the reagent was below 0.28 %, the possibility of the formation of visible crystals was larger.

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THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

X. General Consideration

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It has been known that glass-like crystals occur in canned sea foods. Those crystals are called struvite, of which chemical formula is $MgNH_4PO_4 \cdot 6H_2O$; they are dissolved in acid, and harmless for human beings.

The cause of the formation of the crystals in the canned foods has been studied by many investigators. However, the exact cause was not determined. In this series of papers, the authors have described their studies on the mechanism of the formation of the crystals in test tubes and the obtained experimental results have been demonstrated practically in the processing of canned sea foods.

From many experiments the following results were obtained: chemical components of magnesium and phosphate which are contained in the canned fish meat and ammonium which is generated from the muscle protein are combined in the heating process of the sterilization, the compound crystallizes out in larger size during the slow cooling processing after sterilization and the formed crystals furthermore grow to larger sizes during storage at comparatively high temperature.

The solubility and the shape of the crystals in the test tube are influenced by the temperature. The maximum solubility of the crystal is at about 50°~60°C. But above 50°C, the shape of the crystals is amorphous, while below 50°C, the crystal is needle-shaped having regular faces. In the present discussion the crystallizing temperature in canned foods is below 50°C.

The crystals which generate at 50°~30°C are particularly large and have beautiful regular faces. The authors have called the range of 50°~30°C **"Zone of the formation of large crystals"**. At the cooling of the canned foods after sterilization, when the temperature of the can passes rapidly through this zone, the crystals formed are small and numerous. If the formation of the crystals, even if they are invisible, is inevitable in the canned sea foods, the size of the crystals should be remained as small as possible. For that purpose, rapid cooling should be carried out strictly. However, even the invisible small crystals will grow to larger visible size during storage at as high temperature as 50°~30°C, so the storage temperature also should be lower.

The value of ion pH of canned food has an influence also upon the solubility of the crystal. In the acidic side below pH 4.0, the solubility becomes larger, therefore the crystals do not easily form in the acidic side. As far as the taste of the canned food permits, the addition of acids may be a method for preventing the formation of the

crystals. But in many cases, the addition of acids makes the taste poor.

The ion concentration of the components, magnesium, ammonium and phosphate, will exert influence upon the formation of the crystals. Especially the amount of magnesium is important, because the amount of magnesium is sufficient if there is only one third of the amounts of the other components of the crystal. That is to say, when magnesium is contained above 0.0012%, the visible crystals may possibly form. In many canned sea foods the amount of the contained magnesium is above the amount required to form the crystals. Therefore the formation of the microcrystals is inevitable. The amounts of the components of the crystals in the pickle of the canned foods vary with the kind, the condition of the content and the freshness of the raw material. For example, when the raw material was unfresh, in spite of the increasing of the amount of ammonia, the number of crystals was small. This may be owing to the decrease of the amount of magnesium by the washing of the meat before packing, or by the decomposition of the meat.

It was described in paper I that in such canned foods, mackerel, saury, salmon etc. having high viscous juice, the size of the crystals formed was small. When the juice of the canned foods is high viscous, the crystallization is slow, but if the crystals once are formed, they have possibility to attain large size, and to become to have beautiful regular faces. Therefore, it is impossible to discuss the formation of the crystals in the various kinds of canned foods having various properties of contents only on the basis of the difference of the viscosity of the juice. The fact that there are only a few crystals in canned mackerel or saury is perhaps due to the low concentration of the components in the juice of the large quantity and to its being agitated by the rapid transfer of the heat in the juice during the cooling process. In those respects the condition of the formation of the crystals in canned mackerel or saury is different from that of canned crab.

Thus the various factors influencing the formation of the crystals are considered. Among those factors the most important one is considered to be the temperature and the concentration of the components of the crystals. As the formation of the crystals in the canned sea food is inevitable, the size of the crystals formed should be kept to invisible ones (microcrystals). The fact that the formation of the crystals in canned sea foods is not greatly influenced by the difference between the use of fresh water or sea water as factory water means perhaps that a very small quantity of magnesium is sufficient to form the crystals. Even if fresh water is used, the amount of magnesium in the raw fish material is already sufficient to enable formation of the crystals

However, recently Fujii and Yamada[1] have suggested that the formation of the crystals may be due mainly to the fresh raw material. They have estimated the amount of the crystals in canned foods by separating the crystals by means of the difference of the specific gravities of carbon tetrachloride and sodium iodide followed by determining

the amount of magnesium in the separated crystals. By this method of estimation, they have studied the causes of the formation of the crystals. According to the obtained results, they have suggested (1) that the difference of the cooling velocities did not exert influence upon the formation of the crystals (size and shape), (2) that the using of sea water is not necessarily the cause of the formation of the crystals and (3) that the raw material itself contains the principal cause of the formation of the crystals. In their method for the estimation of the amount of the crystals, they have said that visible crystals (above 0.2～0.3 *cm* ～ 0.1 *cm*) can be separated by using carbon tetrachloride and that crystals of one-tenth the size of the visible crystal can be separated by using sodium iodide. However, in the present authors' experiments, not only visible size crystals, but also invisible size (microcrystals) (13×8 μ) pass into the layer of carbon tetrachloride. To summarize Fujii and Yamada's paper[1] practically reaches the conclusion that the quantity of magnesium of the visible crystals and some part of the microcrystals can be estimated. On trial, the difference of the formation of the crystals owing to the cooling velocities is shown in Table 1.

Table 1. The state of the crystals formed in the course of cooling canned crab by various method (reprinted from Fujii and Yamada's report)

Sample No.	Cooling method	Amount of formed crystals (amount of Mg *mg*)				
		Perchment paper	Meat and juice		Total (*mg*)	Size of crystal on perchment paper
			CCl_4-layer	NaI-layer		
A	Cooled by fan (40 min.)	29.3	48.4	0.1	77.8	large
	Cooled in sea water (6°C, 10 min.)	62.6	47.4	2.0	112.0	middle
B	Cooled by fan (40 min.)	72.6	54.5	31.3	158.4	middle
	Cooled in sea water (6°C, 10 min.)	77.7	49.4	43.4	170.5	middle
C	Cooled by fan (50 min.)	30.3	85.8	24.2	140.3	small
	Cooled in sea water (3°C, 10 min.)	34.3	78.7	19.2	132.2	small
D	Cooled by fan (50 min.)	15.1	123.1	29.3	167.5	small
	Cooled in sea water (3°C, 10 min.)	31.3	119.1	10.0	160.4	middle

In presenting Table 1, Fujii and Yamada have said that there seems to be no difference in the amount and size of the crystals according to cooling velocities. But the present authors would like to comment the results in Table 1 with regard to the cooling velocity, Fujii and Yamada have not estimated the temperature in the can by a thermo-couple. The cooling by electric fan for 40 minutes in Table 1 makes the temperature at the center of the can 50°～45°C, for 50 minutes, 45°～40°C according to the present authors' experiences. When the cans are cooled by being submerged in sea water of 6°

or 3°C for 10 minutes, the temperature will be 35°~40°C. After having been submerged, when the cans are left in the air, the temperature falls very slowly. Their temperature passes very slowly through **"Zone of the formation of large crystals"**. In Fujii and Yamada's experiments, they have not discriminated the cooling velocies at all. So there was perhaps no difference in the formation of the crystals (size and number) in relation to the cooling velocities. However, if the cooling by an electric fan is defined to be **"slow cooling"** and the cooling by submergence in sea water is defined to be **"rapid cooling"**, the results except D-sample showed somewhat smaller sized crystals, or the same on the parchment paper of the canned crab cooled rapidly than that cooled slowly. As the total amount of magnesium in Fujii and Yamada's results may contain the visible and invisible microcrystals as stated above, the only amount of magnesium in the separated crystals which passed into the carbon tetrachloride layer should be compared. If the amounts of the magnesium are compared by cooling velocities, the amount in the cans cooled rapidly in sea water is less than that in those slowly cooled by an electric fan as seen in Table 1. In Fujii and Yamada's results, if the argument is as above, the comparison of the formation of the crystals from the cooling velocities is considered to be possible. According to Fujii and Yamada's results, difference in the formation of the crystals dependent upon freshness of the material was seen. The size of the crystals on the parchment paper and in the juice of the cans made from fresh raw materal, is larger than that from unfresh material. Those results agreed with the present author's results. However, the influence of the cooling velocity upon the formation of the crystals is clearly greater than that of freshness of raw material, according to the present authors' results.

It is advisable to use a complex-making agent in order to prevent the formation of the crystals in the canned foods. The present authors have used various kinds of complex-making agents, and found that the amount of sodium hexametaphosphate should be above 0.11~0.22 % of the content of the canned food, E. D. T. A. of 0.34~0.67 % and Mascolin "M" and "T" ($M_n+2P_nO_{3n+1}$) of 0.1~0.28 % in order to prevent the formation of the crystals. But the addition of any large amount of those complex-making agents exerts a bad influence upon the taste of the food and health of human beings. So the addition of complex-making agent should be less than 0.11~0.22 % of sodium hexametaphosphate and Mascolin "M"., "T".

As noted above, the formation of the crystals, $MgNH_4PO_4 \cdot 6H_2O$, is inevitable in canned marine foods. Therefore the size of the crystals formed should be controlled below visibility by rapid cooling after the sterilization in the retort or by the addition of a complex-making agent in quantity below the point where it would injure the taste or quality of the canned foods. However, it is difficult rapidly to cool the processed cans by a large amount of fresh water on the floating canneries, so the cooling method or the addition of adequate complex-making agent must be further considered in future.

Literature Cited

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ON FISH TROPOMYOSIN

II. Terminal Residue in Tropomyosins

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Bailey[1] has obtained negative results for N-terminal residue in rabbit skeletal tropomyosin and suggested that the tropomyosin is composed a large cyclic peptide chain, together with the report of physico-chemical results.[2] On the other hand, the C-terminal residue in rabbit skeletal tropomyosin has been proved by Locker[3] to possess one isoleucine residue, who used the carboxypeptidase method and the hydrazine method. The C-terminal sequence has been also found to be isoleucine-serine-threonine-methionine-isoleucine-alanine-. It seems to be of interest in respect to protein structure that the number does not coincide in N- and C-terminal residue.

As results of that the amino acid composition has been investigated on tropomyosin from the different types of mammalian muscle, from lower classes of vertebrates and invertebrates phyla. The differences according to animal origin have been remarkably observed in the contents of lysine, arginine and amide nitrogen.[4-7]

In this study, assay of N-terminal residue in fish tropomyosin is carried out by Sanger's DNFB (dinitrofluorobenzene) method[8] and of C-terminal residue by the standard hydrazine method of Akabori and Ohno.[9-12] It is shown that fish tropomyosin contains no N-terminal residue and possesses one isoleucine residue for C terminal residue. In addition to fish, the same results are obtained in bull frog and squid tropomyosin.

EXPERIMENTAL

Protein preparation

Haddock and bull frog tropomyosin, which was purified by repeating the precipitation and salting out as described in previous paper,[7] were crytallized three times, using Bailey's method.[13] Squid tropomyosin is crystallized three times by Yoshimura's method.[4] These tropomyosins dissolved in 0.1 M KCl at pH 7.5 are dialyzed against water at 2°C for two days. Viscous solution obtained after filteration is freezing-dried to fiber. Molecular weight of tropomyosin adopts 53,000 for haddock and bull frog and 54,300 for squid.

Assay for N-terminal residue

The DNFB method is applied for the detection and estimation of N-terminal residue in proteins. Since the most of DNP-amino acids are very sensitive to light in dilute solution,[14] the procedure of all analysis is carried out in dark room.

Haddock tropomyosin is treated with DNFB under the conditions recommended by Sanger.[8] After the DNP-protein is hydrolyzed in sealed glass tube by 6 N HCl at 100°C

for desired time, hydrolysate is extracted by ether to divide ether soluble fraction and water soluble fraction. Amide nitrogen in DNP-protein is determined in order to check amounts of original protein.

Ether soluble fraction: After evaporating ether, dinitrophenol in residue is sublimated under the conditions of 80°C and 1 mm. pressure. This sublimation of dinitrophenol is repeated three times; the ether solution of residue is chromatographed. Each spot, which is separated by two dimensional paper chromatography of Levy,[15] is eluted in 5 cc. of 1 per cent $NaHCO_3$ at 60°C for 15 min. and estimated at 360 mμ. Obtained results are shown in Table 1.

Table 1. DNP-amino acids in ether soluble fraction from molar haddock tropomyosin

DNP-amino acid	Hydrolysis time		
	4 hrs.	8 hrs.	16 hrs.
	(mol)	(mol)	(mol)
Asp or Glu	trace	trace	trace
Ala	trace	trace	trace
Di-Lys	trace	trace	trace
Leu or ILeu	0.05	0.06	0.08

In the detected four types of DNP-amino acid, DNP-leucine or DNP-isoleucine is obtained to the extend of no more than 0.1 mol per molar haddock tropomyosin and in trace for the other DNP-amino acids. The total molar of these DNP-amino acids is only about 0.1 mol per molar protein, these quantities seem to be arisen from some impurities. Four types of ether soluble DNP-amino acid attributable to impurities have been also observed in rabbit tropomyosin.[1]

The results obtained from bull frog and squid tropomyosin at 16 hours hydrolysis are shown in Table 2. In these cases, each DNP-amino acid which seems to be due to impurities, does not reach to 0.1 mol per molar protein.

Table 2. DNP-amino acids in ether soluble fraction from molar bull frog and squid tropomyosin

DNP-amino acid	Bull frog	Squid
	(mol)	(mol)
Asp or Glu	0.02	—
Ala	trace	0.03
Di-Lys	trace	—
Leu or ILeu	0.06	0.05

Values obtained from 16 hours hydrolysate of DNP-protein.

Water soluble fraction: Water soluble DNP-amino acid is detected in 16 hours hydrolysate of DNP-protein by use of paper chromatography, solvent system n-BuOH: CH_3COOH: H_2O = 250: 60: 250[16] and acetone: 3M CH_3COOH = 92: 8,[17] and with silica gel column chromatography, solvent system 66% methylethylketone-ether.[8] The spot or band corresponding to ε-DNP-lysine is merely detected in each tropomyosin.

From these results, no N-terminal residue exists in haddock tropomyosin and the same facts are found in bull frog and squid tropomyosin.

Assay for C-terminal residue

C-terminal residue is detected by the method of Akabori and Ohno[9)-12)] on 20-30

mg. of haddock tropomyosin which is dried over P_2O_5 at 100°C for 4 hours.

Prelimiary test: The protein is hydrazinolyzed with about 1 g. of anhydrous hydrazine in sealed glass tube at 100°C for 10 hours. After the excess of hydrazine is removed in vacuo over conc. H_2SO_4, the residue is dissolved in 5 cc. of water. For the removal of amino acid hydrazide which is formed in resionous precipitate with aldehyde, 0.3 cc. of isovaleraldehyde is added to the solution. Solid $NaHCO_3$ is added to the solution, maintaining pH 7 and stirring occasionaly for 20 min. The precipitate formed is filtered off and washed twice with 1 cc. of water. The removal of amino acid hydrazide is repeated again, and the solution is extracted with 5 and 3 cc. of ethyl acetate. The combined ethyl acetate layer is washed with 3 cc. of water and is rejected. The water phase obtained, about 10 cc. is subjected to dinitrophenylation with 0.3 cc. of DNFB in ethylalcohol 20 cc. and 0.3 g. $NaHCO_3$. After shaking for 2 hours in dark at room temperature, the reaction mexture is diluted with 50 cc. of water. The solution acidified with 2 N HCl is extracted with 30, 20 and 10 cc. of ethyl acetate. The ethyl acetate combined is extracted with 30, 30, 20 and 10 cc. of 2 per cent $NaHCO_3$, and the $NaHCO_3$ solution combined is washed with 30 and 20 cc. of ethyl acetate. Most of DNP-amino acid and a part of isovaleral derivatives of DNP-aspartic and DNP-glutamic mono-hydazides are transferred into the $NaHCO_3$ solution, while all of di-DNP-tyrosine and a part of di-DNP-histidine remain in ethyl acetate layer. Fraction A: the $NaHCO_3$ solution is acidified with 2 N HCl and extracted with 30, 20 and 10 cc. of ethyl acetate. After evaporating ethyl acetat, dinitrophenol in residue is sublimated three times and chromatographed by Levy's method like as in the assay for N-terminal residue. Fraction B: after ethyl acetate layer is evaporated in vacuo, the residue dissolved in 50 cc. of ether is extracted with 30, 20 and 10 cc. of 2 per cent $NaHCO_3$ solution. The $NaHCO_3$ solution combined is acidified with 2 N HCl and extracted with ether. The residue, obtained from ether fraction, is chromatographed by Levy's method.

To ascertain spot separated, the eluted solution of spot is hydrolyzed with 6 N HCl for 4 hours, and rechromatogrpahed. The strong spot of DNP-leucine or DNP-isoleucine and the trace of DNP-alanine, DNP-glutamic acid or DNP-aspartic acid are obtained from only fraction A.

Identification of C-terminal amino acid by Dowex-50 chromatography: Hydrazinolysate of 52.1 mg. haddock tropomyosin is treated with isovaleradehyde as described above, except for the use of pyridine instead of $NaHCO_3$ to adjust pH. Aqueous solution washed with 5 and 3 cc. of ethyl acetate is dried up and the residue is chromatographed by the method of Moore and Stein[18] as shown in Fig. 1. Isoleucine, most positive free amino acid is obtained 0.69 mol per molar protein and for glutamic acid, 0.06 mol, and alanine, 0.04 mol. The strong spot DNP-leucine or DNP-isoleucine found in preliminary test, is due to isoleucine which is in one C-terminal residue of the protein. Other amino

acid, glutamic acid and alanine seem to be caused by impurities.

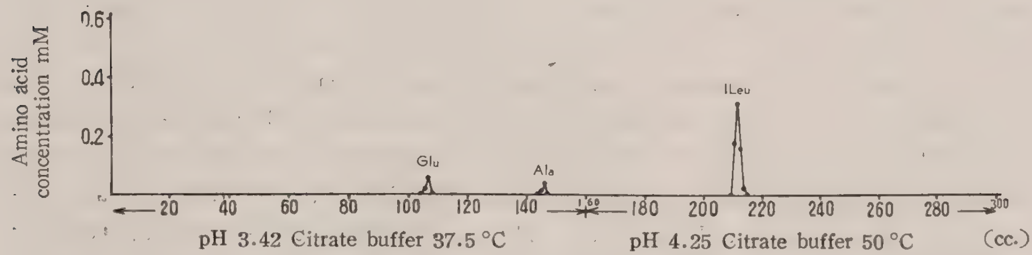


Fig. 1. Elution curves of free amino acids for a 10 hours hydrazinolysis of haddock tropomyosin on a 0.9×100 cm. Dowex-50 (X-8) column (52.1 mg. of protein hydrazinolysate is used, after iso-valeraldehyde treatment)

Estimation of DNP-amino acid from C-terminal residue: Haddock tropomyosin is hydrazinolyzed for 5, 10, 15 and 20 hours, and treated as in preliminary test. Spot separated from fraction A is eluted and estimated at 360 mμ. The results obtained are shown in Table 3. Isoleucine yields about 1 mol per molar protein for every hydrazinolysis time, and seems to be correspond to C-terminal residue for haddock tropomyosin.

Table 3. DNP-amino acids in hydrazinolysate from molar haddock tropomyosin

DNP-amino acid	Hydrazinolysis time			
	5 hrs.	10 hrs.	15 hrs.	20 hrs.
	(mol)	(mol)	(mol)	(mol)
ILeu	0.72	1.00	0.75	0.65
Glu	0.05	0.05	0.04	0.01
Ala	0.02	0.03	0.01	trace

The values of isoleucine are corrected as follows: its recovery yields 65% for 5 hours hydrazinolysis, 65% 10 hours, 62% 15 hours and 55% 20 hours.

Isoleucine has been detected in about 25 per cent of the theoretical amount for C-terminal residue in rabbit tropomyosin,[3] and in the trace amount of glutamic acid, serine, glycine and alanire for impurities. But in this study, the yields of isoleucine are better on 10 hours hydrazination, about 0.65-0.52 mol per molar protein without correction.

In Table 4 is given the estimations of DNP-amino acid on 10 hours hydranization of bull frog and squid tropomyosin, after the performance of preliminary test and of identification by Dowex-50 chromatography. Isoleucine is also detected as C-terminal residue in both proteins.

Table 4. DNP-amino acids in hydrazinolysate from molar bull frog and squid tropomyosin

DNP-amino acid	Bull frog	Squid
	(mol)	(mol)
ILeu	0.81	0.81
Ser	0.03	——
Glu	——	0.04
Ala	trace	trace

Values obtained from 10 hours hydrazinolysates of proteins, and isolucine is corrected as in Table 3.

DISCUSSION

The tropomyosin of haddock, fish, have no N-terminal and one isoleucine for C-terminal residue. The same facts are found in tropomyosin of bull frog, amphibia, and of squid, cephalopoda.

On the amino acid composition of general protein, the specificity of animal origin is remarkable in the contents of neutral and aromatic amino acid, for example, serum albumin,[19] and hemoglobin.[20 21] The differences of terminal amino acid according to animal origin are also observed in these proteins: leucine or alanine corresponds to C-terminal residue for serum albumin[22] and valine or methionine to N-terminal residue for B chain of hemoglobin.[23]

Although there are some specificities to general amino acid composition of tropomyosin, high acidic amino acid, arginine and lysine contents, it is observed regularity that arginine replaces some of lysine[4-6] with alteration from high phyla to low phyla and this replacement is completed in annelid.[6] Fish tropomyosin is distinctly more close to mammalian tropomyosin in general property than to that of invertebrates.[7] But the quantity and the quality of terminal amino acid is the same in rabbit, haddock, bull frog and squid.

It may be considered respecting the molecular pattern of rabbit tropomyosin possibility of cyclopeptide structure having a branch point at glutamic γ- or aspartic β-linkage is caused by the fact of no N-terminal and one C-terminal residue, together with its axial ratio. The same fact, that does not agree in the number of each terminal, has also been observed in pepsin[24] and taka-amylase.[25] In the view of molecular shape, tropomyosin of fish, bull frog and squid may be the same as rabbit's from the results obtained.

SUMMARY

Terminal residue of fish tropomyosin is studies, using DNFB method for N-terminal and hydrazine method for C-terminal residue.

Fish tropomyosin has no N-terminal residue and one isoleucine for C-terminal residue the same as rabbit's. In addition to fish, the same results obtain in bull frog and squid tropomyosin.

The author wishes to express his heartiest thanks to Professor Katsuzi Yoshimura, Faculty of Fisheries, Hokkaido University, for his invaluable advices and constant encouragement throughout the present study, and to Professor Shiro Akabori and his collaborators, Faculty of Science, Osaka University, for their instruction in the technique of terminal analysis.

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結晶鯨 Pepsin

第3報 Paper Chromatography による構成アミノ酸組成の検索

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Studies on Crystalline Whale Pepsin

III. Analysis of amino acid by paper chromatography

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Abstract

The following amino acids were found as the constituents in the whale pepsin by means of paper chromatography of its acid- and alkali- hydrolysates: cystine, aspartic acid, glutamic acid, glycine, serine, threonine, lysine, histidine, arginine, alanine, tyrosine, α-aminobutyric acid, proline, valine, methionine, tryptophane, phenylalanine, and leucines. The pepsin contains α-aminobutyric acid as a specific component.

Between the two whale and swine pepsin, some differences could be found in the relative contents of the constituent amino acids. Whale pepsin showed higher contents for basic amino acids (arginine, lysine, and histidine) in the case of acid hydrolysates and for S-containing amino acids than swine pepsin in the case of alkali hydrolysates.

緒言

前報[1,2]に於て鯨 pepsin か豚 pepsin とは若干性質を異にする事を認めたが，特に至適 pH・等電点・電気泳動的挙動の相異する事より構成アミノ酸組成の比較を試みた、paper chromatographyによるアミノ酸組成の種属特異性検索に関しては，先に Sanger[3]（1949）が豚・牛・羊の insulin に就て serine, glycine, threonine, alanine 含量の相異する事を認めた。（これは後に Lens and Evertzen 1952，Harfenist 1953等の完全分析により確認せられた。）又，安藤・石井[4]（1952）は邦産鰊白子蛋白 clupein 中に北欧産のものに存在しない glycine を特異的に含有する事を示している。更に Tuppy and Paléns[5]（1955）は cytochrome C に就て牛・鮭・鶏間に alanine, serine 含量を異にする事を報告している。

ここに鯨・豚両 pepsin の構成アミノ酸を paper chromatography により検索した所，鯨 pepsin には α-amino butyric acid を特異成分として含有する他，塩基性アミノ酸・含Sアミノ酸の含量の大なる傾向が見られ，その元素組成に於てS-含量の大なる事，等電点の相異する事などに合致する結果が示された。

Moore & Stein 法によるアミノ酸完全分析に就ては次の機会に報告する。

実験の部

試料

鯨 pepsin・豚 pepsin 共に前報[2]と同一結晶試料を用いた。

Paper chromatography

試料の酸分解・アルカリ分解液に就て二次元法で paper chromatography を行い，これを更に水解液の濃

紙泳動・一次元展開法により group separation を行い各 fraction の抽出液に就て特異反応・標準アミノ酸との対比を行つて個々のアミノ酸を同定した。

酸分解—上記結晶 pepsin 50mg に 6 N-HCl 0.8ml. 添加，封管の下 110～115°C 油浴中15～17時間分解，水解液を湯浴上濃縮，蒸発乾固，再蒸溜水で溶解，濾過して humin 質その他不溶性残渣を除去，再び蒸発乾固，これを繰返して可及的酸を除去後中和・濾過・濃縮・蒸発乾固後 0.5ml. の蒸溜水に溶解して供試。以下能う限り定量的に操作した。

アルカリ分解—結晶 pepsin 50mg に10倍量の飽和バリタ水を加え封管の下湯浴内 4 時間水解後稀 H_2SO_4 で中和してバリウムを除去，濾液を湯浴上濃縮，少量の再蒸溜水で溶解，これを 3 回繰返して最後に 0.5ml. 蒸溜水に溶解し供試，以下前同様出来る限り定量的に操作した。

展開—東洋濾紙 No.50 (40cm×40cm) を用い一端より 5 cm× 5 cm の所に試料 0.2ml. を附し 21～23°C の下で上昇法によつた。溶媒は一次に *n*-butanol：酢酸：水（容量比 9 ： 1 ： 7 の上層），二次に phenol：水（容量比 4 ： 1 ）を用いそれぞれ約22時間及27時間（約30cm）展開後乾燥，0.25% ninhydrin acetone溶液を噴霧，100°Cに加熱発色した。

Group separation —— 二次元法で検出せられた各アミノ酸を更に確認する為に次の様にして group separation を行い各 spot の抽出液に就て標準アミノ酸との対比及特異反応により同定した。即ち，巾15cm の前記濾紙片を用い前記 phenol ：水を溶媒として一次元展開を行い一端を ninhydrin 溶液で呈色せしめた所大体 5 group に分離せられたので，予想呈色部に対応する個所を切取り各部を細刻，水及微アルカリ液で抽出，この両抽出液に就て上記両溶媒を別々に用いて一次元展開を行い，夫々に就て標準アミノ酸と対比同定した。この中第 2 ・ 3 group には酸性・中性・塩基性アミノ酸の混在が見られたので濾紙泳動により更に各 spot を分別した。即ち，東洋濾紙 No. 50 (25cm× 8 cm)を用い citrate buffer (pH 4.5) を溶媒として 450volt 20mA 2 時間通電後気乾，一端を切取り ninhydrin で発色，spot の予想存在部位を見究めて更に 3 group に分ち各々を上と同様に溶出並に再度両種溶媒による一次元展開により同定した。尚 valine と methionine 分別は phenol : *m*-cresol（溶量比 1 ： 1 ）を borate buffer (pH 9.3) で飽和したものを溶媒とした。又 leucine と isoleucine は分離せぬが達摩形の spot により両者を識別した。

特異反応——両溶媒につき一次元法による展開後乾燥し夫々の map に特異反応試薬の噴霧により発色，標準アミノ酸と対比同定を行つた。即ち， tyrosine, tryptophane には phenol 試薬， proline 及 oxyproline には isatine 反応，arginine 並に lysine には坂口反応， histidine には *p*-bromoanilin 反応，cystine 及 methionine には塩化白金酸試薬，arginine と lysine 並に cystine には更に sod.nitroprusside 試薬，serine 及 threonine には sod.periodate 飽和の Nessler 試薬により同定した。

実験結果並に考察

鯨 pepsin の HCl 及バリタ水解液の二次元展開 paper chromatogram はそれぞれ第 1 図及第 2 図に示す如くである。同様処理して得られた豚 pepsin の paper chromatogram と比較して定性的には α-amino butyric acid 以外には差が認められない。α-amino butyric acid に就ては田中等[6] (1953) がアコヤ貝成分 conchiolin 中に同様 paper chromatography で存在を認め，これがバリタ水解液中に比較的顕著に存在し且つバリタ濃度を40%以上に上げるとその量が増大する傾向を示す事により水解による二次的分産物である事の可能性を見ており，一般にアルカリ水解に際し threonine, glutamic acid よりの二次的生成が認められているものではあるが，全く同様に処理した豚 pepsin 水解液には存在せず，鯨 pepsin では酸，アルカリ両水解液に認められる事，又鯨眼房水中[7] 及海綿の spongine 中[8] にもその存在が認められているので鯨 pepsin の特異成分として一応首肯される所と考える。

次に両 pepsin の paper chromatogram を比較して，spot の呈色度及呈色範囲より判断して第 2 表の如きアミノ酸含量の傾向が見られた。即ち，酸分解液に就ては明かに塩基性アミノ酸含量が鯨 pepsin に多く，

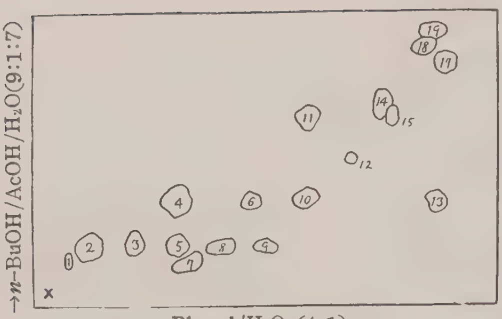


Fig. 1. Paper chromatogram of amino acids in acidhydrolysate of whale pepsin

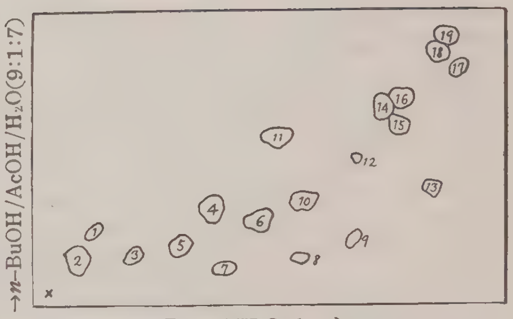


Fig. 2. Paper chromatogram of amino acids in alkali hydrolysate of whale pepsin

First phase: phenol/water (4:1). Second phase: *n*-butanol/acetic acid/water (9:1:7, upper layer). Ninhydrin development : 1, cystine; 2, aspartic acid; 3, ghutamic acid; 4, glycine; 5, serine; 6, threonine; 7, lysine; 8, histidine; 9, arginine; 10, alanine; 11, tyrosine; 12, α-amino butyric acid; 13, proline; 14, valine; 15, methionine; 16, tryptophane; 17, phenylalanine; 18, leucine; 19, isoleucine.

Table 1. Amino acid constituents of whale and swine pepsin

Amino acid	Acid-hydrolysis		Alkali-hydrolysis		Methods of identification
	Whale pepsin	Swine pepsin	Whale pepsin	Swine pepsin	
1 Cystine	+	+	++	±	Reaction of hydrochloroplatinic acid & reaction of sod. nitroprusside
2 Aspartic acid	++(+)	+++	++(+)	+++	Fractionation by ionophoresis & then Rechromatography
3 Glutamic acid	++	++	++	++	
4 Glycine	+++	+++	++(+)	++(+)	
5 Serine	++	++	++	++	Frationation by ionophoresis Reaction of sod. periodate
6 Threonine	++	++	+++	+++	
7 Lysine	++	+	+(+)	+	Frationation by ionophoresis Reaction of Sakaguchi & reaction of sod. nitroprusside for arginine & lysine Reaction of *p*-bromo amilin for lysine
8 Histidine	++	+	+	±	
9 Arginine	+(+)	+	+	±	
10 Alanine	++	+(+)	++	++	Elution & rechromatography
11 Tyrosine	++	++	++	++	Folin's reaction
12 α-Amino-butyric acid	+	−	+	−	Elution & rechromatography
13 Proline	++	++	++	++	Reaction of isatine
14 Valine	++	++	++(+)	++(+)	Buffered phenol : *m*-cresol solvent
15 Methionine	+	+	+(+)	+	Reaction of sod. nitroprusside & reaction of hydrochloroplatinic acid
16 Tryptophane	−	−	++	++	Folin's reaction
17 Phenylalanine	++	++	+(+)	++	Elution & rechromatography
18 Leucine	++	+++	++	++(+)	
19 Isoleucine	++	+++	++	++(+)	

又アルカリ分解液に於ては含 S-アミノ酸含量の多い傾向を示している。一方，豚 pepsin は aspartic acid, valine, isoleucine 及 leucine 等が多い。cystine はアルカリ分解に不安定とされているが，この水解条件下ではアルカリ分解の方に強い呈色反応を示した。

ここに電気泳動的挙動並に元素組成と一致する傾向を示す結果を得たが，更に全アミノ酸の完全分析によりこれを確認する積りである。

要　　約

結晶鯨 pepsin 及豚 pepsin のアミノ酸組成を paper chromatography で検索した所，鯨 pepsin には α-amino butyric acid が存在し，且つ塩基性アミノ酸及含 S-アミノ酸 (cystine, methionine) 含量が豚 pepsin に比し多い事が認められた。

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結晶鯨 Pepsin

第4報　各種蛋白消化液中の生成遊離アミノ酸検索

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Studies on Crystalline Whale Pepsin

IV. Free amino acids in peptic digests of various proteins

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Abstract

Casein, edestin, egg-white, zein, insulin (whale), and γ-globulin were hydrolyzed with whale pepsin or swine pepsin. The kinds of free amino acids liberated during the process and the degree of liberation were examined by paper chromatography. The results, as shown in Tab. 1-3, confirmed that the amino acids liberated by both pepsins were of large variety, and that in the case of whale pepsin the liberation of amino acids at pH 3.0 was higher than that at pH 1.8 qualitatively or quantitatively. Proteolysis type of whale pepsin is fairly different from that of swine pepsin.

緒　　言

Protease の種属差判定には Bergmann 一派の如く各種合成 peptides を用いる方法とは別に，天然蛋白質を基質とするときは Green & Neurath (1954) の言の如く 'enzymatic denaturation' 等の如き一般 peptide 結合の解裂とは異る作用の特異性も観察される利点がある。

一方，蛋白質の pepsin 消化における遊離アミノ酸の生成は頭初は否定せられていたのであるが[1)-4)]，その後多数の研究者により相当種類の遊離アミノ酸が検出されている。即，Felix[5)] (1925) は histone より Lys, Northrop[6)] (1930) は pepsin の自己消化により Tyr, Damodaran[7)8)] (1932) は edestin より Tyr, Asp (NH_2), gliadin より Glu (NH_2), Jones & Gersdorff[9)] (1933) は casein より CyS, Lieben & Lieber[10)] (1934) は casein 及 gelatin より Arg, Calvery *et al.*[11)12)] (1936) は結晶 egg albumin より Tyr, Try, Cys, Hankes *et al.*[13)] (1948) は casein より Arg, 福田[14)] (1953) はegg albumin, casein, gelatin 等より多種類のアミノ酸，Denton & Elvehjem[15)] (1953) は牛肉・casein・zein より相当量の Arg, 少量の Thr, Val, Leu 等，以上何れも遊離アミノ酸として検出している。

ここに各種天然蛋白質を基質として時間と分解能の推移，及生成遊離アミノ酸の検索により豚・鯨両 pepsin の類属差を観察した。分離生成物の検索には peptides をも対象とすべきであるが，先ず遊離アミノ酸に限定してこれを一定条件下 paper chromatography で検索し両 pepsin の作用を比較した。

最近，構造の判明している insulin の A-chain, B-chain を基質として protease の特異性を観察する事が行われており，鯨 insulin の両 peptides に対する鯨 pepsin の分解態度は Sanger の行つた豚 pepsin とは明かに異るのであるが次の機会に報告する予定である。

試料及方法

試料：前報[16)]と同一の結晶鰮鯨 pepsin と豚 pepsin の1% 'stock solution' (60%glycerin の $^{N}/_{1000}$ HCl 溶液)

蛋白質：casein (Hammarsten) — Merck 製

結晶 edestin — 前回の結晶標品

凝固卵白 — 局法試験法に準じ調製

zein — corn meal より Mason & Palmer[17] の alcohol 法により調製

鯨 insulin — 当教室で調製した鰮鯨の精製標品

結晶 γ-globulin — 馬血清より Na_2SO_4 法により分離結晶化せる結晶標品，武田薬工研究所大村栄之助氏より恵与。

消化試験：蛋白消化には各基質を2〜3%濃度に N/100 HCl 30ml. に溶解或いはsnspendし pHを調節後(稀酸又は稀アルカリ) 基質300部に対し酵素量1部の割で添加，少量の toluol を加えて後35°Cにて消化，一定時間毎にVan Slyke の amino N を測定して全Nに対する分解度を pH 1.8 と 3.0 に就て観察（卵白は生成 amino N量で表示）した。分解進行につれて pH が変化する場合があるのでその都度 pH を調節した。

Paper chromatography：消化液 10ml. を取り alcohol を加えて80%濃度とし未消化蛋白及 peptides を除去後 desiccator 中真空濃縮，以下前回に準じてこれを再蒸溜水で温浸出して不溶物を去り湯浴上蒸発乾固，再び蒸溜水で溶出して中和後更に蒸発乾固，最後に 0.2〜0.5ml. 蒸溜水で溶解供試。東洋濾紙 No. 50 (40 cm×40cm) を用い展開温度 20〜23°C に於て上昇法（約30cm）で展開。溶媒は一次に phenol：水（容量比４：１），二次に *n*-butanol：醋酸：水（容量比９：１：７上層）を使用。発色には 0.25% ninhydrin acetone 溶液を噴霧。分離不良及確認困難なる時は前報[18] に準ずる方法によつた。

実験結果並に考察

A. Casein 消化

Casein に就て豚，鯨両 pepsin の pH1.8 並に 3.0 における分解度の時間的推移と生成遊離アミノ酸は第1図及第1表に示す如くである。外観上の変化は豚 pepsin は速やかに懸濁状の casein 粒子を液化透明（pH1.8 では約30分，pH 3.0 では約60分）とするも鯨 pepsin では液化進度小，且全体を半透明にする程度に終る。この際のこの溶解能は凝固卵白の場合同様 pH 1.8 の方が3.0 よりも若干強い。即ち，外観上の変化（消化速度）は豚 pepsinの方が明かに消化力大，且つ両pepsin 共に pH 1.8の方が 3.0よりも作用大。

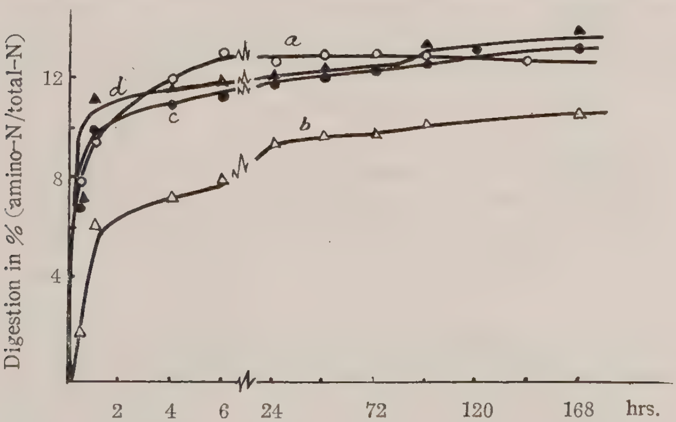


Fig. 1　Hydrolysis of casein

Curve a: Digestion of swine pepsin on casein at pH 1.8, 35°C

Curve b: Digestion of swine pepsin on casein at pH 3.0, 35°C

Curve c: Digestion of whale pepsin on casein at pH 1.8, 35°C

Cuive d: Digestion of whale pepsin on casein at pH 3.0, 35°C

分解度

豚 pepsin：分解の初速度極めて大でpH 1.8では約6時間で平衡状態に達するがpH 3.0では漸増の傾向を示す。分解度は常に明かに pH 1.8の方が大でその差は反応初期には大差を示すもその後時間経過と共に次第に減少する。

鯨 pepsin：約1時間で殆ど平衡に達し以後極めて徐々に分解進行す。pH 3.0 及 1.8 共に類似の分解度を示すが前述の如く外観上の液化力は pH 1.8 の方が大なるに拘らず分解度は小差で pH 3.0 の方が大。

Table 1. Kinds of free amino acids in peptic digests of casein

Amino acid	pH 1.8				pH 3.0			
	Swine pepsin		Whale pepsin		Swine pepsin		Whale pepsin	
	Beg.	*End*	*Beg.*	*End*	*Beg.*	*End*	*Beg.*	*End*
Leu (Ilen)	+	++	−	++	+	+	+	++
Val	−	+	−	−	−	−	−	−
Met	+	+	−	−	−	−	−	−
Try	+	+	−	±	−	−	−	±
Tyr	±	+	−	+	+	+	+	+
Ala	−	+	−	+	+	+	−	−
Thr	+	+	−	−	−	+	+	+
Ser	−	+	−	±	−	−	+	+
Gly	+	+	+	+	−	−	−	+
Glu	+	+	−	+	−	+	−	+
Asp	+	+	−	−	−	−	−	−
Arg	+	++	−	+	−	−	−	+
His	−	+	−	−	−	−	−	−
Lys	±	+	−	−	−	+	+	+

生成アミノ酸

豚 pepsin：第1表に示す如く pH 1.8 と 3.0 における分解に於て，初期では Leu (Ileu), Tyr が共通でその他 pH 1.8では Met, Try, Thr, Gly, Glu, Asp, Arg, Lys, pH 3.0 では Ala が認められる。明かに pH 1.8 に於ける peptidase 作用大。分解末期になると両者の差接近し Leu (Ileu), Tyr, Ala, Thr, Glu, Lys が共通でその他 pH 1.8 に於て Val, Met, Try, Ser, Gly, Asp, Arg, His が認められる。即ち，pH 3.0 に於ては分解度が小なるに応じて生成遊離アミノ酸は定性的にも定量的にも明かに劣る。

鯨 pepsin：pH 1.8 と 3.0 では分解同程度なるに拘らずアミノ酸生成は大差を示し(特に反応初期に於て)，pH 3.0 では Leu (Ileu), Tyr, Thr, Ser, Lys の生成が認められるに拘らず pH 1.8 では単に Gly のみで大部分の分解生成物は peptides なる事を示す。分解末期には両 pH に於ける差接近するも Ala, Thr, Lys の生成に於て相互に差異を認む。

両 pepsin を比較する時は pH 1.8 では分解度類似するも豚 pepsin の方が，pH 3.0 に於ては分解度に応じて鯨 pepsin の方が，それぞれアミノ酸生成量大，全般を通じて豚 pepsin のみが生成するものとして Val, Met, Asp, His が挙げられるに対し鯨 pepsin のみが生成するアミノ酸は無く，豚 pepsin の方が広範囲の分解を行う事を示す。

B. Edestin 消化

Edestin 消化の結果は第2図及第2表に示す。完全に溶液状態に在る為この場合は外観上の変化は認められない。

分解度

豚 pepsin：pH 1.8 では約3時間で一旦平衡に達するも120時間を経過して再び上昇す。pH 3.0 でも同様3時間で急速な初期分解を終るが以後引続き漸増す。両 pH に於ける分解度は1時間消化では差極めて小なるも以後はかなりの差を示して pH 1.8 の方が大，分解末期には再び両者の分解度接近するに至る。

鯨 pepsin：分解の初期・末期を通じて明かに pH 3.0 の方が分解度大（3時間以後は約2倍以上の大差，）且つ共に1週間を経過しても平衡に達せずに尚分解進行す。両 pepsin の分解度を比較する時は，初期では鯨 pepsin の方稍々大，次で豚 pepsin の方がかなり大となり末期には再び鯨 pepsin の方 (pH3.0) 大となる。

生成アミノ酸

豚 pepsin：第2表に示す如く分解初期に於ては両 pH に於ける遊離アミノ酸は Met, Ala, Asp (NH_2) が

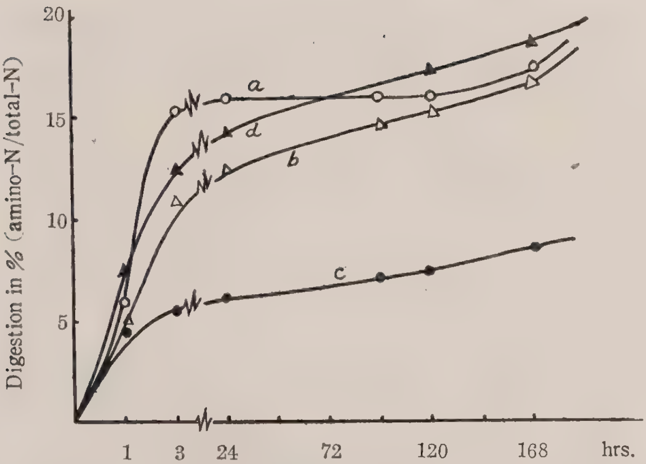


Fig. 2 Hydrolysis of edestin

Curye a: Digestion of swine pepsin on edestin at pH 1.8, 35°C
Curve b: Digestion of swine pepsin on cdestin at pH 3.0, 35°C
Curve c: Digestion of whale pepsin on edestin at pH 1.8, 35°C
Curve d: Digestion of whale pepsin on edestin at pH 3.0, 35°C

共通でその他 pH1.8ではLeu (Ileu), Thr, Lysが，pH3.0では His の生成が見られる。末期になると共通アミノ酸成分としては Leu (Ileu), Met, Ala, Ser, Asp (NH_2), Arg, His となりこの他 pH1.8では Phe, Tyr, Thr, Lys が挙げられ，初期末期共に pH 1.8の方が生成量大，前例の鯨pepsin の casein 消化の場合の如くに両 pH に於ける分解度類似するも，豚pepsin では pH 1.8 の方がアミノ酸生成量 (peptidase 作用) 大である。

鯨 pepsin : pH 1.8 並に 3.0に於ける分解度の差大なるも遊離アミノ酸生成には余り差が認められない。即ち，分解初期では Leu (Ileu), Met, Tyr, Ala が共通成分として，この他 pH 1.8では Ser が見られる。末期に

Table 2. Kinds of free amino acids in peptic digests of edestin

Amino acid	pH 1.8				pH 3.0			
	Swine pepsin		Whale pepsin		Swine pepsin		Whale pepsin	
	Beg.	*End*	*Beg.*	*End*	*Beg.*	*End*	*Beg.*	*End*
Leu (Ileu)	+	++	±	++	−	+	±	++
Phe	−	++	−	−	−	−	−	+
Met	±	+	±	±	±	±	±	+
Tyr	−	+	±	+	−	−	+	+
Ala	++	++	+	+	±	+	±	+
Thr	+	+	−	−	−	−	−	+
Ser	−	+	±	±	−	+	−	±
Asp (NH_2)	+	+	−	−	+	+	−	−
Arg	−	+	−	−	−	+	−	+
His	−	+	−	−	+	+	−	−
Lys	+	+	−	−	−	−	−	−

なると pH 3.0 に於ける生成量増大し Phe, Thr, Arg が出現する。

両 pepsin を比較するに，pH 1.8 では分解度の差に応じて豚 pepsinの方が遙かに遊離アミノ酸の生成多く，pH 3.0 に於ては両 pepsin の分解度の差小で生成アミノ酸量も差少いが Phe, Tyr, Thr, Asp (NH_2), His 生成が相互に異る。全般を通じて豚 pepsin のみが生成する遊離アミノ酸として Asp (NH_2), His, Lys が挙げられ 鯨pepsin にはかかるアミノ酸無し。casein の場合同様に鯨 pepsin の方が作用範囲狭し。

C. 卵白消化

i）熱凝固卵白

実験結果は第3表及第3図に示す如くである。外観的の変化は20—30分で豚 pepsin は卵白粒子を消化し透

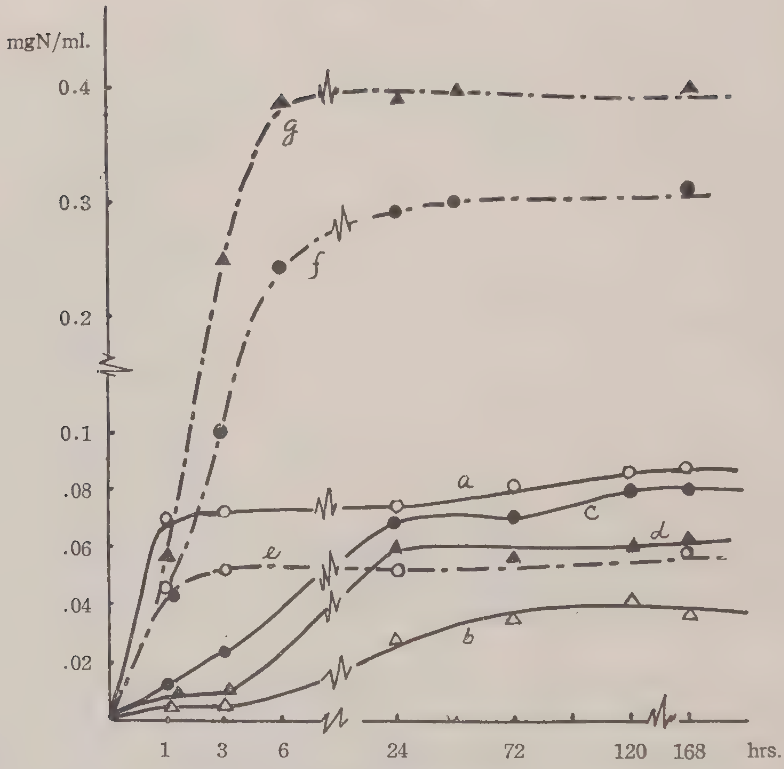


Fig. 3 Hydrolysis of egg-white.
Curve a: Digestion of swine pepsin on heat coagtd. egg-white at pH 1.8, 35°C
Curve b: Digestion of swine pepsin on heat coagtd. egg-white at pH 3.0, 35°C
Curve c: Digestion of whale pepsin on heat coagtd. egg-white at pH 1.8, 35°C
Curve d: Digestion of whale pepsin on heat coagtd. egg-white at pH 3.0, 35°C
Curve e: Digestion of swine pepsin on raw egg-white at pH 1.8, 35°C
Curve f: Digestion of whale pepsin on raw egg-white at pH 1.8, 35°C
Curve g: Digestion of whale pepsin on raw egg-white at pH 3.0, 35°C

Table 3. Kinds of free amino acids in peptic digests of egg-white

Amino acid	Heat coagulated egg-white								Raw egg-white			
	pH 1.8				pH 3.0				pH 1.8		pH 3.0	
	Swine pepsin		Whale pepsin		Swine pepsin		Whale pepsin		Swine pepsin		Whale pepsin	
	Beg.	*End*	*Beg.*	*End*	*Beg.*	*End*	*Beg.*	*End*	*Beg.*	*End*	*Beg.*	*End*
Leu (Ileu)	±	+	−	−	−	+	−	+	±	+	+	+
Val	−	+	−	−	−	−	−	+	−	−	−	+
Tyr	+	+	±	+	±	±	−	−	−	±	−	+
Ala	−	±	−	+	−	±	+	+	±	±	−	−
Thr	−	−	−	−	−	−	−	−	−	−	+	+
Ser	−	+	−	−	−	−	−	−	−	−	−	+
Glu	+	+	−	−	±	±	−	+	−	−	−	−
Arg	−	±	−	−	−	−	−	−	−	±	−	−
His	−	±	−	−	−	−	−	−	−	−	−	−

明な溶液とするが鯨 pepsin は1時間後も尙相当量の卵白粒子が残存する。これをそのまま長時間消化するも半透明のままで透明な液となる事なし。共に pH 1.8 の方作用大。

分解度

豚 pepsin：pH 1.8 に於ては1時間までに急速に，以後は極めて緩慢な分解経過を示す。pH 3.0 に於ては約3時間位までの初期分解は pH 1.8 より遙かに劣るもその後次第に増加し72時間以降平衡に達しその差約½程度となる。

鯨 pepsin：小差を以て常に pH 1.8 に於ける分解度大で液化力と相応す。又分解初速度極めて緩慢なるも24時間以後はかなりの分解度となる。分解初期に於ける proteinase 作用小なるも二次的分解能が大きい為であろう。

両 pepsin を比較する時，共に pH 1.8 に於ける分解度の方が大。反応初期では豚 pepsin の pH 1.8 に於ける分解度が圧倒的に大きいが時間経過と共に両 pepsin の差減少するに至る。

生成アミノ酸

豚pepsin：反応初期では pH 1.8 並に 3.0 に於ける生成遊離アミノ酸は Tyr, Glu を共通とする他 pH1.8 に於て Leu (Ileu) が少量見られるのみで，分解度の差は極めて大きいがこれには余り差異を認めない。pH 1.8 に於けるこの期の分解反応が主として proteinase 作用である事を示す。分解末期では Leu (Ileu), Tyr, Ala, Glu が共通で更に pH 1.8 では Val, Ser, Arg, His が見られるが，分解の初期，末期共に定性的にも定量的にも前述の edestin や casein に劣る事が認められる。

鯨 pepsin：分解度小なる事から予想される如く遊離アミノ酸は極めて少く反応初期に於て pH1.8 では Tyr pH 3.0 では Ala の各々1種類を生成するのみ。末期には pH 3.0 に於ては peptidase 作用若干進行する事が認められる。この場合でも proteinase 作用は pH 1.8 の方が大なるに拘らずアミノ酸生成量は pH 3.0 の方が大なる事を示している。

両 pepsin を比較する時は，分解度に相応して豚 pepsin の方が初期・末期共にアミノ酸生成量大，而して共に pH 1.8 に於ける分解度の方が大なるに拘らず豚 pepsin は pH 1.8，鯨 pepsin は pH 3.0 に於けるアミノ酸生成量が大である。全般を通じて豚 pepsin のみが生成するアミノ酸として Ser, Arg, His が挙げられ前同様に鯨 pepsin よりも広範囲の分解を示している。

ii）生卵白消化

実験結果は凝固卵白消化と共に第3図及第3表に示す。外観的の変化は初めから僅かに白色の沈澱を生成するのみで以後の変化は認められない。

分解度

豚 pepsin：約3時間で平衡に達す。凝固卵白より若干分解能劣り，且つ変性蛋白の方は24時間以後も漸増の傾向にある。

鯨 pepsin：約1時間までの初期反応では豚 pepsin と同程度なるも以後急速に分解進行し豚 pepsin の約8倍の分解能を示すに至る。約6時間で平衡に達する。この場合は凝固卵白と異り常に pH 3.0 に於ける分解度の方が大である。

両 pepsin を比較し，豚 pepsin は変性卵白の方が消化能大なるに対し鯨 pepsin が生卵白の方が遙かに消化能大なる事は注目に値する。専ら生食を行つている鯨の食餌性から見て当然の事と思われるが，蛋白質分解の第1段階に於ける 'enzymatic denaturation' 作用が特に強いのであるか，又は蛋白質の 'native form' に対し特別に親近性を有する二次構造を持つている為か――興味を惹く問題である。

生成アミノ酸

豚 pepsin：pH 1.8 に於ける遊離アミノ酸生成は熱変性蛋白よりもその分解度小なるに相応して相当劣る。即ち，変性卵白からは Leu (Ileu), Val, Tyr, Ala, Ser, Glu, Arg, His を生成するのに対し Leu (Ileu),

Tyr, Ala, Arg を認めるに過ぎぬ。

鯨 pepsin： pH 3.0 に於ける遊離アミノ酸生成量は熱変性蛋白に比し分解度大差を示すに拘らず，それ程の差異が認められない。主として peptides 生成の分解反応である事を示している。但し熱変性卵白と生卵白の分解では Tyr, Ala, Thr, Ser, Glu 等の生成に於て相互に異り分解型式が大きく相異する事を示している。

両 pepsin を比較する時 pH 1.8 に於ける豚 pepsin と pH 3.0 に於ける鯨 pepsin のアミノ酸生成能では鯨 pepsin の方が定性的にも定量的にも優るが分解度の差程ではない。全般を通じて豚 pepsin のみが生成するものとして Ala, Arg, 鯨 pepsin のみが生成するものとして Val, Thr, Ser が見られ相互にかなり分解の様相を異にしている。又これ迄の例と異り鯨 pepsin の方が作用範囲大である。

D. Zein 消化

Zein 消化に関する結果は第 4 図及第 4 表に示す如くである。外観上の変化は豚 pepsin の pH 1.8 に於ける分解末期にはかなりの液化が認められ，その他は何れも蛋白粒子は不溶解のままなるも鯨 pepsin はこの場合 pH 1.8 に於ける方が稍々優る。

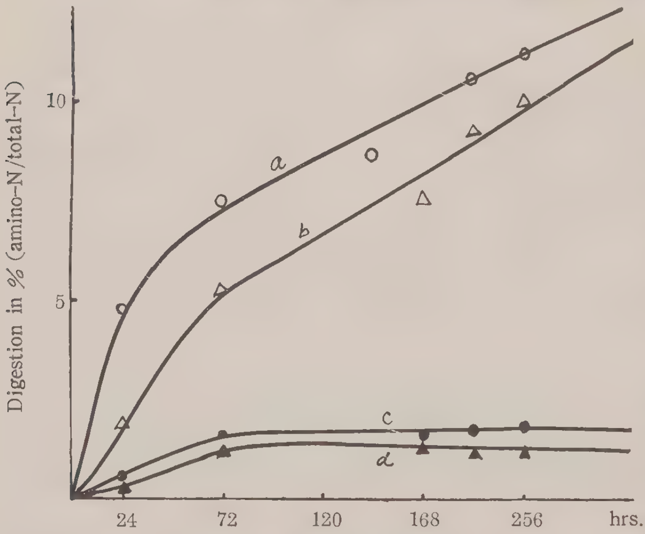


Fig. 4 Hydrolysis of zein
Curve a: Digestion of swine pepsin on zein at pH 1.8, 35°C
Curve b: Digestion of swine pepsin on zein at pH 3.0, 35°C
Curve c: Digestion of whale pepsin on zein at pH 1.8, 35°C
Curve d: Digestion of whale pepsin on zein at pH 3.0, 35°C

分解度

豚 pepsin：外観上の変化に相応して pH 1.8 に於ける分解度大。これ迄の例と異り 200 時間を越えても分解上昇を続け平衡状態に入らぬ。

鯨 pepsin：殆んど分解せざるも外観上の変化に相応して小差で pH 1.8 の方が分解度大である。豚 pepsin と異り約 70時間で平衡に達す。

両 pepsin を比較して，豚 pepsin が分解の初速度は遅いが casein, edestin 同様に相当分解するのに対して鯨 pepsin は分解能極めて小である。これは鮪 pepsin[19] が zein を分解し難いとの報告が見られるが，鯨 pepsin が同一環境に棲む魚類の pepsin とその substrate

Table 4. Kinds of free amino acids in peptic digests of zein

Amino acid	pH 1.8		pH 3.0	
	Swine pepsin	Whale pepsin	Swine pepsin	Whale pepsin
	End	*End*	*End*	*End*
Leu (Ileu)	++	±	+	+
Val	++	−	+	+
Tyr	+	±	±	+
Ala	+	−	−	−
Ser	+	−	+	−
Gly	±	−	±	+
Glu	+	−	±	+
Arg	±	−	−	−

specificity を同じくしている事を示す。

生成アミノ酸

豚 pepsin：両 pH に於けるアミノ酸生成を見ると Leu (Ileu), Val, Tyr, Ser, Gly, Glu を共通成分としてこの他 Ala, Arg が pH 1.8 に於て見られる。何れも良く生成するが定量的には遥かに pH 1.8 の方が大で先の分解度に相応した結果を示す。

鯨 pepsin：両 pH に於ける遊離アミノ酸を比較して Leu (Ileu), Tyr が共通でこの他pH3.0では Val, Gly, Glu が認められ，分解度は小差で pH 1.8 の方が大なるもアミノ酸生成量は遥かに pH 3.0 の方が多い。

両 pepsin を比較して Leu (Ileu), Val, Tyr, Gly, Glu の共通成分の他に豚 pepsin のみが生成するものとして Ala, Ser, Arg が挙げられ分解範囲豚 pepsin の方が大きい事を示す。但し分解度に於ける程の大差を示さないのは鯨 pepsin が 'one by one' の分解型式を取る為と思われる。

不溶性蛋白である zein, 凝固卵白，或いは casein 粒子に対する液化作用が豚 pepsin 大いに優り，鯨 pepsinは分解第1期に於けるこの proteinase 作用は極めて弱いが二次的分解は比較的大きい。即ち，豚 pepsin は α-amylase, 鯨 pepsin は β-amylase に類似した分解型式を示す事になる。

E. 鯨 Insulin 消化

分解度

試料の関係で168時間の分解物に就てのみ観察。

豚 pepsin：pH 1.8に於ける分解度13.4%を示し pH 3.0に於ける8.2%に比しかなり大きな差が認められる。

鯨 pepsin：pH 3.0 に於ける分解度 13.1% で pH 1.8 に於ての 7.4% に比べて約2倍の分解能を示す。この様に溶性蛋白に対する消化能は鯨 pepsin では常に大差で pH 3.0 の方が大きい。

生成アミノ酸

第5表に示す如く両 pepsin 共に分解度に相応して豚 pepsin では pH 1.8，鯨 pepsin では pH 3.0に於て生成量大。これを定性的に見ると Leu (Ileu), Val, Tyr, Ala, Ser, Glu を共通成分とし，この他豚 pepsin では Asp, 鯨 pepsin では Gly を生成し，定量的には Leu (Ileu), Val, Tyr, Ala 等に於て差を示し，相互に若干分解態度を異にする事が認められる。

Table 5. Kinds of free amino acids in peptic digests of purified whale insulin

Amino acid	pH 1.8		pH 3.0	
	Swine pepsin	Whale pepsin	Swine pepsin	Whale pepsin
	End	*End*	*End*	*End*
Leu (Ileu)	+	−	±	±
Val	±	−	−	++
Tyr	+	±	++	±
Ala	+	−	−	++
Ser	±	−	−	±
Gly	−	−	−	+
Glu	+	±	±	+
Asp	+	−	−	−

F. γ-Globulin 消化

試料の関係で時間と分解度の推移は観察しなかつたが168時間の分解では pH1.8に於ける豚 pepsin は9.2% pH3.0 に於ける鯨 pepsin は 10.1%と略同程度の分解度を示す。

生成アミノ酸

pH1.8に於ける豚pepsin, pH3.0に於ける鯨pepsin消化の結果は第6表に示す如く，分解度に相応して共に良く遊離アミノ酸を生成す。共通成分の他に豚pepsinのみが生成するものとしてAla, Serが，鯨pepsinのみが生成するものとしてTyr, Thrが見られ，又定量的にはLeu (Ileu), Met, Glu, Aspに於て差を示し相互にかなり分解態度を異にする事が認められる。

Table 6. Kinds of free amino acids in peptic digests of γ-globulin

	pH 1.8 Swine pepsin	pH 3.0 Whale pepsin
	End	*End*
Leu (Ileu)	+	++
Val	+	+
Met	+	±
Tyr	−	±
Ala	+	−
Thr	−	±
Ser	+	−
Glu	+	±
Asp	++	±

G. 対照実験

同一実験条件でpH1.8又は3.0に於ける基質蛋白よりのninhydrin陽性物質は認められない。又pepsin試料よりはninhydrin陽性spot (Tyrその他) 2〜3認められるも実験と同一条件の二次元展開では全く検出せられぬ程度の微量である。

要 約

Casein, edestin, 卵白, zein, 鯨insulin, γ-globulin等各種蛋白のpH1.8並に3.0に於ける分解度と時間の推移，及生成遊離アミノ酸をpaper chromatographyで検索し，豚pepsinと鯨pepsinの分解型式を比較し，相互にかなり異る事を認めた。

終りに貴重な結晶γ-globulinを御恵与された武田薬工研究所大村栄之助氏に厚く謝意を表す。

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結晶鯨 Pepsin

第5報 変性と Activity との関係

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Studies on Crystalline Whale Pepsin

V. Relation between denaturation and activity

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Abstract

1. Effect of urea or guanidine on the activity of whale pepsin.

As demonstrated in Tab. 1, a marked loss of enzymic activity is noticed if whale pepsin is exposed to urea or guanidine. After being stored in 5.0 M urea at 40°C. for 10 hours, the proteolytic activity was reduced to 40% of its initial value; whereas when stored in 8.0 M urea for 5 hours or 3.0 M guanidine for 20 hours complete inactivation resulted.

It may be concluded that, unlike ribonuclease, whale pepsin does not retain activity after treatment with urea or guanidine. Moreover, the rate of inactivation of whale pepsin by the denaturants is much higher than that of swine pepsin.

2. Digestion of denatured proteins with whale pepsin.

As shown in Figs. 1 ～ 3, the ability of whale pepsin to digest urea-denatured hemoglobin or heat-coagulated fish meat is much inferior to that of swine pepsin; however, the ability to digest raw fish meat of the former is much superior to that of the latter. The proteolytic activity of whale pepsin on denatured proteins was reduced to 30–50% of its value for raw materials.

緒　　言

前報[1]では鯨 pepsin の pH と安定度との関係に就て観察し豚 pepsin とは pH の安定域をかなり異にする事を示したが，ここに変性処理に対する挙動を基質の側と酵素の側とより両 pepsinの性質を比較し，尿素・グアニヂン変性に対する安定度が若干異なる事，又専ら生食を行つている鯨の食餌性から見て基質蛋白の熱変性・尿素変性による消化能への影響は興味を惹く所であるが，両 pepsinを同一条件下に観察し鯨 pepsinの未変性蛋白に対する消化能の大なるを認めた。

原則的に protease は‘denatured’ form を良く消化する事は周知の所，鯨 pepsinが魚類pepsin 同様に‘native’ form を良く分解する事は，水解初期に於てこれら熱変性・尿素変性とは性質を異にする様な denaturase 作用が特に大きいのであろうか？

試料及方法

鯨 Pepsin

前報[1]の鰮鯨 (*Balaenoptera borealis* Less.) の結晶 pepsin 3回再結。

豚 Pepsin

Worthington Chemical Sales Co., New Jersey, U.S.A. 製のもの3回再結。

Activity 測定

前報同様 Anson 法[2]に準じ消化液の TCA 溶存部の Folin 値を比色定量。

Pepsin の尿素・グアニヂン処理

3.0~8.0M 尿素乃至はグアニヂン含有の pH4.4 の acetate buffer に pepsin を溶解（最終濃度0.3%）40°C に放置後一定時間毎に activity 測定（基質は hemoglobin）。

消化試験

牛 hemoglobin 調製及その尿素変性処理・activity 測定は共に Anson法[2] に準ず。

魚肉消化は鰮・イカの筋肉部を肉挽器で磨細せるもの 2g につき水 20ml. を加え HCl で pH 調節後 pepsin は 0.1% 'stock solution' 1.0ml. 添加35°C 60分間消化後 Anson の hemoglobin 法同様に処理し TCA溶存部に就て光電比色計で比色定量。熱変性は100°C 10分間加熱により行つた。pH測定は前回同様硝子電極pH-meter によつた。

実験結果並に考察

I. 鯨 pepsin の尿素 並にグアニヂン変性

豚 pepsin に対する尿素変性の影響に就ては Steinhardt[3]（1938）が 3 °Cの低温下では1.0~6.0M の濃度に於て15日間にわたり activity が保持される事を認めているが，一方 Perlman[4]（1956）によると，8.0M 濃度で37°C 24時間で全く activity 喪失，25°C では40% を残存するのみなる事を示し，比較的高温下ではかなり不安定である事を述べている。

ここに豚，鯨両 pepsin の尿素及グアニヂン変性による activity への影響を同一条件下で観察した結果，第1表に示す如く明かに activity の低下を来し，この点 ribonuclease[5] とは明瞭に態度を異にする。両 pepsin 共に尿素よりもグアニヂンによる影響の方が大であるが，何れも鯨 pepsin の方が変性の程度大きく，尿素は 3.0M 60時間では約30%減，5.0M 60時間では全く喪失，8.0M では5時間で殆んど喪失するに至る。グアニヂンでは3.0M 20時間で既に喪失してしまうが豚 pepsin は60時間でも約20%残存す。

Table 1. Dependence of pepsin activity on time of exposure to urea or guanidine at 40°C

Conc. of denaturants used			Relative specific proteolytic activity							
			hr. 0	3	5	10	15	20	30	60
Urea	3.0M	Swine pepsin	100%	98	95	91	88	87	84	80
		Whale pepsin	〃	95	90	88	83	80	75	68
	5.0M	Swine pepsin	〃	86	74	64	60	43	32	18
		Whale pepsin	〃	69	58	48	37	25	15	0
	8.0M	Swine pepsin	〃	50	13	0				
		Whale pepsin	〃	30	2	0				
Guanidine-HCl	3.0M	Swine pepsin	〃	92	88	83	80	64	60	20
		Whale pepsin	〃	80	64	46	25	0		

これに関して Haurowitz *et al.*[6]（1944），Steinhardt[3]（1938），Burk *et al.*[7]（1930）等は hemoglobin の尿素変性・アルカリ変性が由来する動物の種により異にする事を述べているが鯨 pepsin は豚 pepsin よりもこれら変性剤の作用を受け易い二次構造を有する様に思われる。

II. 基質蛋白質の変性と鯨 pepsin 消化能との関係

一般に pepsin,[8,9] trypsin,[11)12] papain[13]（これは特に著しい差を示す）等の protease は何れも天然蛋白よりも変性蛋白質の方が分解度大とされ，これは酵素の inhibitor の熱による破壊の場合を除いて，変性作用により基質蛋白分子の 'unfolding or uncoiling' が行われ基質・酵素両分子の接触可能性増大がその理由と考えられ，従つて 'native' form が分解される場合も一旦変性されてから分解するものとされる。これに就て Christensen[9]（1952）は ovalbumin が pepsin によりよく分解されるが trypsin では分解されないのに対し，β-lactoglobin は逆に trypsin で良く分解されるが pepsin にはあまり分解されない事（変性後は良く分解されるに至る）を示し，ovalbumin は低い pH で β-lactoglobin は高い pH でそれぞれ変性されやすい事よりこれを説明している。

然しながらこの関係は酵素の種類により，又同一酵素に対しても蛋白質の種類によりその程度を異にし，例えば Hanrowitz[11]（1945）は trypsin について ovalbumin・serumglobulin は差大，fibrinogen・myosin 等は差小なる事を認め，豚 pepsin については Christensen[10]（1955）が egg albumin の熱変性の場合は両者殆んど同一なるも，これの尿素変性の場合反応初期に於ては約 50% も変性蛋白の方が消化能大なる事を示し，変性の方法による差も認められこの問題の複雑性が想像される。

ここに鯨・豚両 pepsin について同一基質を同一条件下で消化試験を行い基質蛋白の変性と pepsin 消化度との関係を観察した。

a. 尿素変性 hemoglobin

Hemoglobin は edestin 等と共に強酸による変性速度大で pepsin の作用域では当然酸による変性状態に在るわけであるが，Anson 法の尿素変性処理により第1図に示す如き大きな相違が両 pepsin の間に見られた。即ち，豚 pepsin に於ては強酸側の消化度低下し，反対に pH 3～4 の消化能上昇して opt. pH は1.8から 3～3.5と移動，且つ尿素変性により消化能の上昇（約50% 増）が見られるのに対し，鯨 pepsin では強酸性側の消化能喪失して 'narrow curve' を与え，豚 pepsin とは逆に尿素変性により消化能の低下（約50% 減）が認められる。この場合，反応液中に尿素が存在する故に酵素自身の尿素による影響も考慮しなければならぬが，35°C 10分間の消化初速度の測定なるを以て，主として基質側の変性処理による構造の変化に起因するものと考える。

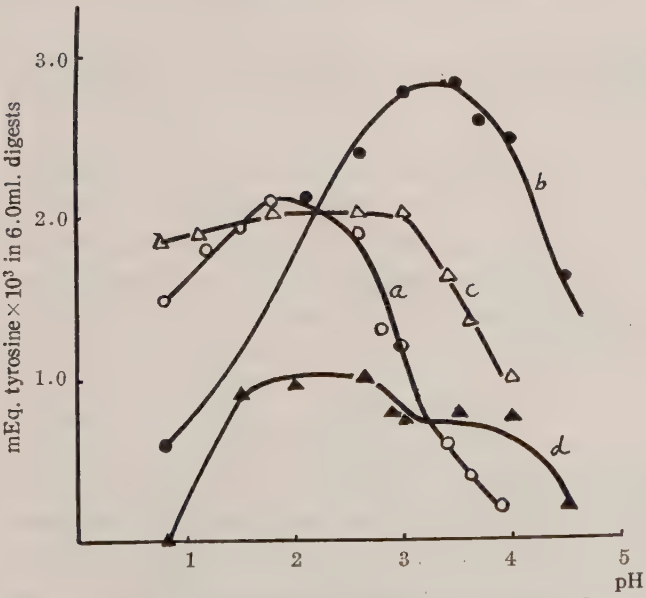


Fig. 1 Hydrolysis of hemoglobin at various pH for 10 min., 35°C
Curve a: swine pepsin on native Hb.
Curve b: swine pepsin on urea-denatured Hb.
Curve c: whale pepsin on native Hb.
Curve d: whale pepsin on urea-denatured Hb.

b. 熱変性魚肉

イワシ及イカ肉に就て生と熱変性せる場合の消化能はそれぞれ第2図並に第3図に示す如くである。

イワシ肉の場合，鯨 pepsin は生の方が約35%消化度大且 opt. pH 2～3 の 'broad curve' を与えるのに対し豚 pepsin では熱変性せる方が約10%消化度大で opt. pH 1.5～2.0を示す。熱凝固せるものに対して鯨 pepsin は

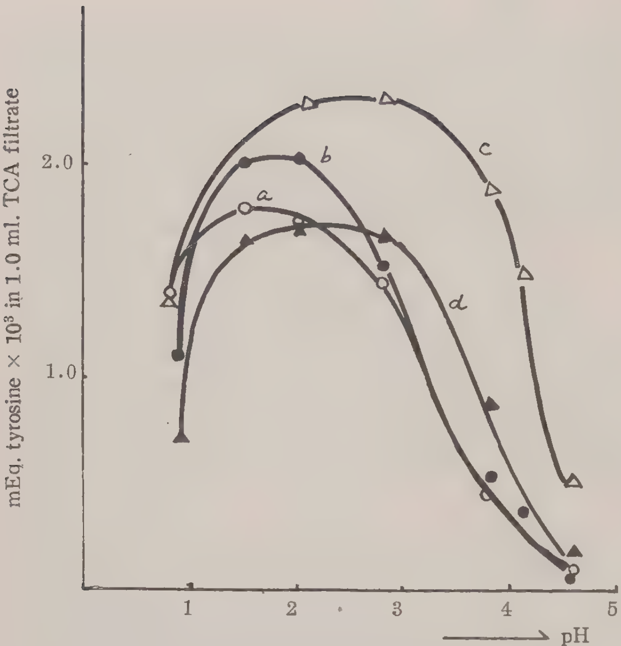


Fig. 2 Hydrolysis of sardine meat at various pH for 60 min., 35°C
Curve a: swine pepsin on native meat
Curve b: swine pepsin on heat-coagulated meat
Curve c: whale pepsin on native squivd meat
Curve d: whale pepsin on heat-coagulated meat

相当劣るも（但し pH 3 以上では稍優る）生肉に対しては豚pepsinよりも遙かに消化能大で特に pH 2～4 に於ける差が大きい。

イカ肉の場合はイワシ肉とは肉質の状態が生・熱凝固の何れに於ても多少異る為両 pepsin共に消化能若干劣るが大体同様の傾向を示す。即ち鯨 pepsin は生肉の方が約 50%大で opt. pH 2～3 の 'broad curve' を与えるのに対し，豚 pepsin では生・凝固共に同程度の消化能（イワシ肉のときより差小となる）で opt. pH 1.8～2.0を示す。この場合も熱凝固せるものに対しては鯨 pepsin は豚 pepsin に劣るも（但しpH3以上では稍優る）生肉に対しては遙かに優る消化力を示す。

一般に魚類 pepsinは蛋白の加熱凝固により消化能低下する事が認められている。即ち，古く Young (1899), Decket (1887) 等は煮沸凝固蛋白が pike胃 pepsinにより極めて消化し難い事を報じているのを始めとして Hammarsten[14] はnative fibrin を pike 胃 pepsin が速やかに消化するも，加熱変性せる fibrin 及凝固卵白は極めて消化し難い事を示し，Bodansky & Rose[15] は種々の魚胃 pepsin が gelatine を容易に消化するも凝固卵白は分解し難い事，高橋・広沢[16] はウナギの胃 pepsin に就てイワシ肉が加熱凝固により著しく消化し難くなる事（対照に用いた腸 protease はあまり差なし）を示し，何れも加熱凝固蛋白の消化し難い事を認めている。

鯨 pepsin も図示の如くこれら魚類 pepsin 同様加熱凝固により消化能低下す（対照の豚 pepsin は 'native' form ≦ 'denatured' form なるに対し)。

Linderström-Lang[17] の schema $N \rightleftharpoons D \xrightarrow{E}$ hydrolysis products

よりすると鯨 pepsin は魚 pepsin 同様 'native' protein に対する denaturase 作用大なるべし。

Christensen (1952)[9] (1955)[10] によると蛋白質の酵素水解の第1歩は 'native' 分子の 'uncoiling' に始まるが，これは単なる 'denatured' form とは若干異る（例えば coiling-up arrangement 維持に重要な特殊な peptide bonds の解裂などを伴う）ものなる事を想像しているが，鯨 pepsin の場合，尿素変性・熱変性とは異る型式の denaturase 作用が行われるものであろう。或いは 'native' form の方に直接接触しやすい構造を有するものであろうか？

要 約

1. 尿素並にグアニヂン変性の鯨 pepsin activity に対する影響は豚 pepsin よりも大で，尿素に於ては 3.0

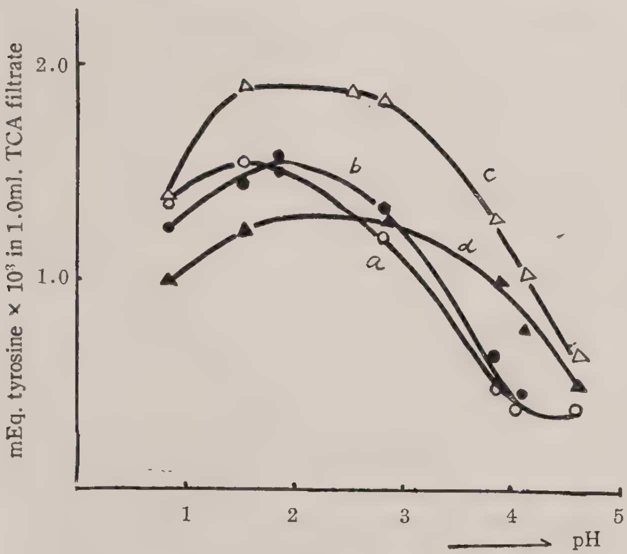


Fig. 3 Hydrolysis of squid meat at various pH for 60 min., 35°C
Curve a: swine pepsin on native meat
Curve b: swine pepsin on heat-coagulated meat
Curve c: whale pepsin on native meat
Curve d: whale pepsin on heat-coagulated meat

M 60時間で約30%減，5.0M 60時間で全く喪失，8.0M では10時間で喪失してしまう。グアニヂンに於ては3.0M 20時間で activity 全く喪失するのに対し豚 pepsin は60時間でも約20%残存す。

2. 基質蛋白の変性と鯨 pepsin 消化度との関係は，hemoglobin に於ては尿素変性により分解度約½となり且つ強酸性側に於ける activity 全く喪失するに対し，対照の豚 pepsin では hemoglobin の尿素変性により消化度約50%増大し且つ opt. pH 3.0〜3.5へ移動す。魚肉消化に於ては加熱凝固により消化能約30〜50%減少し魚類 pepsin 同様 'native' form の消化能大なるを認めた。(対照の豚 pepsin では 'native' form ≦ 'denatured' form)。

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結晶鯨インシュリンに関する研究
第3報 アミノ末端並びにカルボキシル末端

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Studies on Crystalline Whale Insulin
III. N- and C-terminal residues

Yasuzô IIÔ

Abstract

The dinitrofluorobenzene method and the hydrazine method have been applied to the identification and quantitative determination of N- and C-terminal residues of whale insulin respectively. Alike as in other insulins (ox, sheep, and pig), phenylalanine and glycine were found to be N-terminal residues, and alanine and asparagine to be C-terminal residues.

From quantitative studies, the minimum molecular weight of whale insulin was estimated as 5,900.

緒言

牛インシュリンのアミノ末端に就ては，1935年 Jensen[1]がヒダントイン法で Phenylalanineであると報告しているが，定量的でなく且つ又Phenylalanineだけと云う事に疑問があつた。次いで1945年来，Sanger[2)3)4)]が DNFB を用い牛，豚，羊のインシュリンのアミノ末端として Phenylalanine と Glycine とを定量した。当教室では，先に DNFB を用いて結晶鯨インシュリンのアミノ末端を Paper chromatography により定性試験を行い，牛，豚，羊等と同じく Phenylalanine と Glycine である事を認めたが[5]，今回充分量の試料を調製し得たので，更にこれを定量的に検討した。

DNP-アミノ酸の定量には従来 Sanger の行つた Silica gel chromatography が広く用いられているが，種々の見地より著者は Amberlite IR-112を用いる方法[6,7]に従つて行つた。

カルボキシル末端に就ては，酵素的及び化学的の二方法があるが，酵素法では牛インシュリンについて，Lens(1949)[8]がカルボキシペプチダーゼを作用させ Alanineを見い出したが，他に Glycine, Valine, Leucine, Tyrosine, などの痕跡を認めている。其の後，Harris (1952)[9]は同一方法で Alanine, Asparagine, Aspartic acidを検出し，更に Sanger (1953)[4]らもこの酵素をアセチルインシュリンに作用させ，Alanine, Asparagine, Aspartic acidを認めた。其の後鯨インシュリンに就ても同一の結果を報告した(1956)[10]。一方化学的方法として Fromageot (1950)[11]らは $LiAlH_4$ で Glycine と Alanine を，又 Chibnall と Rees (1951)[12]は $LiBH_4$ で Glycine と Alanine とを検出した。更に赤堀等 (1952)[13]はヒドラジン法で Glycine と Alanine とを検出している。当教室では先に結晶鯨インシュリンのカルボキシル末端を赤堀等と同様ヒドラジン法にて定性試験を行い Alanine, Asparagine, 及び α-Aminobutyric acid を検出した[5]。今回著者はヒドラジン法と DNFB 法を併用し Amberlite IRC-50 にて定量を行い Alanine, Asparagine を C-末端基として確認した。

鯨インシュリンに就て末端基の定量的な報告が未だないのでここに報告する。

実験の部

試料，前報[14]の当教室調製イワシ鯨（*Balaenoptera borealis* LESSON）の結晶インシュリンを2回再結を

繰返した。

【A】アミノ末端及び遊離アミノ基

DNP － インシュリンの調製

100mg の再結晶インシュリンと 100mg の $NaHCO_3$ を 5 ml の水に溶かし，10ml の C_2H_5OH と 0.5ml の DNFB を加え，2 時間振盪し，生じた DNP － インシュリンの黄色沈澱を遠心分離後，水，エタノール，及びエーテルで洗滌し乾燥した。このものの Amido－N は 0.96% であり，同様操作で行なつた結晶鯨インシュリンでは 1.34% であつたので，100mg の DNP－インシュリンは 71.5mg のインシュリンに相当する。

末端基の定性

10mg の DNP－インシュリンを 10ml の 20%HCl で 8 時間煮沸し，冷却後，エーテルで 3 回抽出，エーテル区分を水で洗滌し，洗液は水溶液区分に合した。エーテル区分はエーテル除去後，極く微量のエーテルに再び溶かし Paper chronatography により（溶媒，ブタノール：酢酸・水，9：1：7 混合物上層，とフェノール・水，4：1 混合物），DNP-Glycine と DNP－Phenylalanine を確認した。水溶液区分は乾固後，更に 8 時間 20% HCl で加水分解し，常法に従い HCl を除去し，少量の水に溶かし上記溶媒を用いて Paper chromatography を行い，ε-DNP-Lysine を確認した。

末端基の定量

各 20mg の DNP－インシュリンを 10ml の 20% HCl で 8 時間，4 時間煮沸し，冷却後，各々エーテルで 3 回抽出し，エーテル区分は水で洗滌し，洗液は水溶液区分に合した。エーテル区分はエーテル除去後 1% HCl・酢酸（8：2）の混液を溶媒として Amberlite IR-112（H-型 0.9cm×15cm）カラムにて溶出分析を行つた。又水溶液区分は濃縮乾固後，更に 20% HCl にて 8 時間加水分解を行い Amberlite IR-112（Na-型 0.9 cm×15cm）カラムにて M/40 クエン酸緩衝液を溶媒として溶出分析を行つた。それぞれの定量結果は第 1 表に示す通りである。

Table 1. The contents of N-terminal amino acids and lysine of whale insulin

Time of hydrolysis	DNP-amino acid	Estimated values No. of DMP-amino acids per mole of insulin	Recovery factor	Corrected values No. of DNP-amino acids per mole of insulin
Hrs.			%	
8	DNP-Gly	0.43	40	1.07
	DNP-Phe	0.75	74	1.01
	ε-DNP-Lys	0.75	89	0.84
4	DNP-Gly	0.46	57	0.81
	DNP-Phe	0.65	87	0.75
Average	DNP-Gly			0.94
	DNP-Phe			0.88

【B】カルボキシル末端及び酸性アミノ酸

末端基の定性

10mg の結晶インシュリンに 0.5ml の無水ヒドラジンを加え，10時間煮沸，冷却後，硫酸デシケーター中で濃縮乾固，5 ml の水に溶かし，これに 0.3ml のベンズアルデヒドを加え過剰のヒドラジンを除き，濾液を濃縮しこれを極く微量の水に溶かし Paper chromatography により Aspasagine, Alanine, α-Aminobutyric acid の 3 つを認めた。

末端基の定量及び酸性アミノ酸の定量

カルボキシル末端並びに酸性アミノ酸の定量[16)17)]にはアルデヒド処理をしてヒドラジッドを除去する方法

とアルデヒド処理をしない方法とがあり，前者は Tyrosine, Tryptophane, 及び Histidine が末端にある場合，後者は特に酸性アミノ酸のペプチド中の結合状態を調べるのに適している。鯨インシュリンの末端は定性試験で上記3種のアミノ酸を認めていろので後者の方法に従つて定量を行つた。即ち 10mg の再結晶インシュリンに 0.5ml の無水ヒドラジンを加え，10時間煮沸後，DNP 化及びアルカリ抽出を行い，C末端区分を得，これを Amberlite IRC-50（H型 0.9cm×30cm）にて分別し，各 Band を切り取りアセトン・水（1：1）の混合液にて溶出し 360mμ にて比色定量した。次にヒドラジッド区分即ち2％重曹に抽出されずに残つた酢酸エチル層を減圧で乾固し，6N HCl で5時間加水分解して生じた DNP-アミノ酸を酢酸エチルで抽出し，Amberlite IRC-50（H型 0.9cm×30cm）で分離して，DNP-Aspartic acid, DNP-Glutamic acid を定量した。測定結果は第2表に示す。

Table 2. The contents of C-terminal and acidic amino acids of whale insulin

Amino acid residues	State of combination	Estimated values No. of amino acid residues per mole of insulin	Recovery factor	Corrected values No. of amino acid residues per mole of insulin	Total number[14] of moles
Ala	C-terminal	0.86	90 %	0.92	
Asp(NH₂)	C-terminal	1.04	93	1.11	3
Asp(NH₂)	peptide form	1.46	78	1.88	
Glu(NH₂)	peptide form	2.25	78	2.89	7
Glu					
Amide					6

考　　察

第1表に示す通り鯨インシュリンのアミノ末端としては Glycine 及び Phenylalanine を各1箇持ち，分子中に Lysine が1箇含まれている事が確認された。この結果は従来の牛，豚，羊等の結果と全く同一である。

第2表に示す通り鯨インシュリンのカルボキシル末端としては Alanine 及び Asparagine を各1箇持ち，インシュリン分子中には Aspargine 2箇と Glutamic acid 7箇のうち3箇がアマイドとして存在し，4箇が -COOH をもつて存在していることが確認された。これらの結果は牛，豚，羊等と全く同一である。なお定性試験で検出された α-Amino butyric acid が定量的な操作で認められなかつたのは第2報に於て報告したアミノ酸分析でも認められなかつた事から考えて二次的生成物であると考える。既往に於て化学的方法を用いた場合[11,12,13] Glycine が Alanine, Asparagineの他にカルボキシル末端として報告され，酵素を用いる場合[4,8,9,10]には，これが認められないことから問題となつているが今回の場合も DNP-Glycine 区分の痕跡を Amberlite IRC-50 のカラム上で認めたので，この部分を溶出しアンモニヤにより DNP を分離し，Paper chromatography にて再び確認した。反応の際に Glycine を C-末端にするような特別に切れやすい場所があるのかもしれないが詳細は明らかでない。

Table 3. Minimum molecular weight of whale insulin calculated from terminal amino acid residues

Terminal amino acids	Minimum molecular weight
Gly	5,869
Rhe	6,183
Ala	6,590
Asp (NN₂)	5,260
Average	5,975

これら両末端基の定量値より鯨インシュリンの最少分子量を求めると第3表に示す通りである。

即ち，鯨インシュリンの最少分子量は約 6,000 である。なお過去に於て牛，豚，羊等のインシュリンに就いて Harfenist & Craig (1952)[17], Sanger (1955)[18], Sluyterman (1955)[19], Fredericq (1956)[20], (1957)[21] 等が得られた結果も約 6,000 であつた。

要　　約

結晶鯨インシュリンのアミノ末端並びにカルボキシル末端を Amberlite IR-112 及び IRC-50 によるクロマトグラフイで定量し豚，牛，羊等陸上動物のインシュリンと全く同一である事を認めた。

本実験遂行に当り御指導を賜つた斎藤恒行教授，石原義雄助教授に対し深甚の謝意を表す。

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REPORT FROM THE "OSHORO MARU" ON OCEANOGRAPHIC AND BIOLOGICAL INVESTIGATIONS IN THE BERING SEA AND NORTHERN NORTH PACIFIC IN THE SUMMER OF 1955

IV. Diatom Standing Crops and the Major Constituents of the Populations as Observed by Net Sampling [1]

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I. Introduction

On the 1955 cruise of the training ship "Oshoro Maru" to the Bering Sea sampling of plankton by vertical haul for upper 50 meter zone with a fine mesh net (0.112 mm in mesh aperture) was carried out covering almost all parts of the Bering Sea except north of 60 degrees north latitude, though stations of sampling were distributed rather sparsely (Fig. 1). Detailed descriptions on gear, methods, sampling stations and other

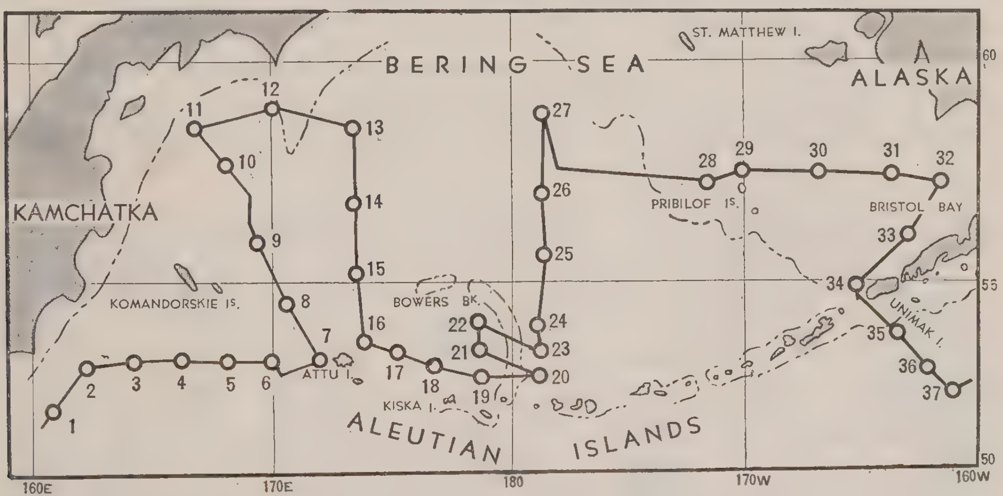


Fig. 1. Chart showing the location of the stations occupied by "Oshoro Maru" during June and July, 1955

records are given in the introductory notes of Motoda & Fujii (1956). The data of rough estimation on the displacement volume, weight and pigment content of the samples in these collections are presented in *Data Record of Oceanographic Observations and Exploratory Fishing*, No. 1, 1957 (pp. 88-92).

In the present studies the author intends to make more exact quantitative estimation of diatom standing crops by counting the cell number, and also to report the characteristic

1) おしよろ丸北洋調査報告 No.8 (昭和30年度)

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species which occupy the main constituents of populations.

The author is gratefully indebted to Prof. S. Motoda, Asst. Prof. T. Kawamura and Mr. M. Anraku for their painstaking guidance throughout the present studies. He expresses his sincere gratitude to Mr. Y. Kawarada, Hakodate Marine Observatory, for his kindness not only in making available his unpublished data but in giving valuable criticism on the present paper. He also sincerely thanks Captain T. Fujii and his crew members as well as the research staff aboard the "Oshoro Maru" on that cruise for their work in sampling at sea. Cordial thanks are also due to Asst. Prof. H. Koto for his generosity in offering his unpublished data on the hydrographic analysis of this cruise.

II. Standing Crops of Total Diatom Populations

As the net used was not equipped with a flow meter at its mouth, the exact quantity of water filtered by its cloth is unknown. All of the data on cell number presented in the present report are those which are multiplied by four on the basis of assumption that such type of the net would allow as little as 25 per cent of the water column to pass through the bolting cloth (Motoda *et al.*, 1957). Moreover, the increase of hauling distance due to the drift of the ship caused by wind may have added to the height of water column. This error was corrected on the basis of the actual records of revolutions of current meter which was attached near the net (see *Data Record*, No. 1, p. 89).

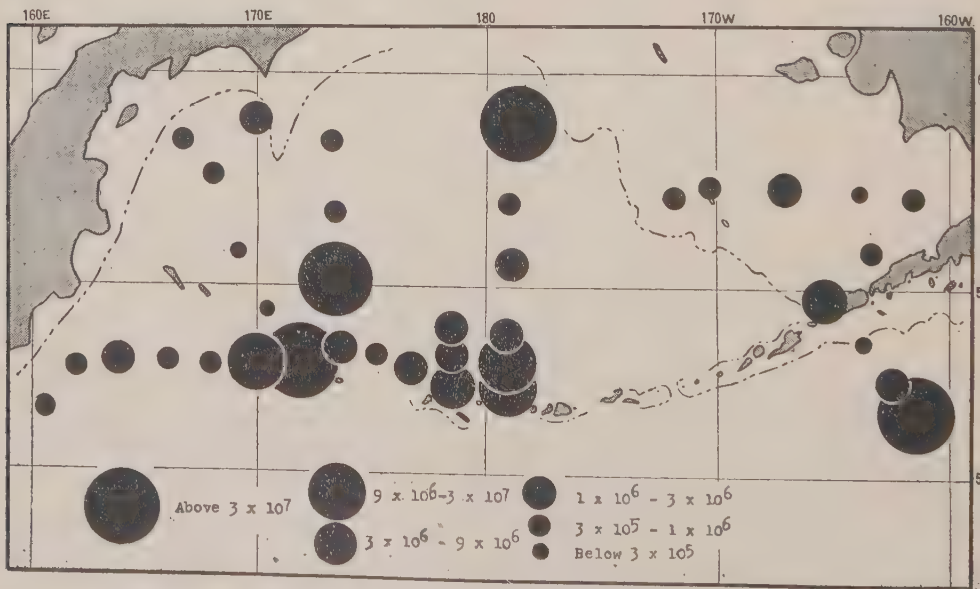


Fig. 2. Abundance of total diatom populations

The full data of the present studies are given in Table 2, among which abundance of total populations without regard to the species composition is illustrated in Fig. 2.

Throughout the whole areas investigated the quantity of diatoms vary from 100000 to 80000000 cells per 1 m^3 of water. Motoda & Kawarada (1955) gave an account of the quantity of diatoms on the basis of similar net samples in the western Aleutian waters taken on the cruise of May-June, 1953. Their data are not corrected either for errors due to filtration coefficient of the net nor for increase of hauling distance in rough weather. For comparison of size of populations between the 1953 and 1955 cruises the value 0.25 is tentatively adopted for filtration coefficient also to the data of 1953 cruise. Thus, the comparion of data from the two cruises shows that there were generally larger diatom populations in 1953 than in 1955.

	1953 cruise (Western Aleutian waters)	1955 cruise (Western Bering Sea: St. Os 1–Os 27)	1955 cruise (All stations)
Largest	494,160,000	79,600,000	79,600,000
Least	30,000	74,800	74,800
Mean	44,384,000	9,870,600	8,456,000

There are found rich populations of diatoms near Attu Island (St. Os 6 & Os 7), east of Bower's Bank (St. Os 20 & Os 23). Also diatoms are rich at St. Os 15, Os 27, Os 34 and at the south-easternmost station out of the Bering Sea (St. Os 37).

Locality	Cell number / m^3
Near Attu Island (St. Os 6 & Os 7)	14,056,000–79,600,000
East of Bower's Bank (St. Os 20 & Os 23)	19,700,000–20,880,000
St. Os 15	55,088,000
St. Os 27	31,920,000
St. Os 34	6,956,000
St. Os 37	33,324,000

Previous report also shows diatom-rich water in the east of Bower's Bank, and considerably too, near Attu Island (Motoda & Kawarada, 1955).

Quantitative data on the diatoms in the Aleutian waters have been presented by Aikawa (1932, 1935). His data are based upon the samples collected with Kitahara's fine mesh net, and no correction for the error regarding filtration coefficient is made. He reported cell number per 1 m^3 of water as follows:

Year	Largest	Mean
1928	Over 3,000,000	935,000
1933	〃 1,000,000	803,000

Although the comparison is very rough in exactness, the above data indicate far smaller populations than the present data. According to the dip-water samplings of Allen (1929,

1930), quantities of diatoms at the surface in Alaskan and Aleutian waters are as follows:

Locality and year	Cell number per m^3 in maximum catch among the stations
Alaskan waters (1923-24)	over 100,000,000
Mary I., Revillagigedo Channel, Alaska (1929)	40,000,000

Cupp (1937) recorded 28560000 cells per m^3 on average for five years (1927, 1929–32) at Scotch Cap Light, Unimak Island. In her summer collection the diatom cells were fairly abundant compared with the present data on St. Os 34 and Os 35 adjacent to Unimak Island.

III. Species Composition of Diatom Populations and its Regional Characteristics

The diatom species which most predominantly appear in the present samples are as follows:

Chaetoceros convolutus
Ch. debilis
Ch. seiracanthus
Ch. constrictus
Rhizosolenia hebetata f. *semispina*
Rh. heb. f. *hiemalis*
Denticula sp.
Ch. concavicornis
Ch. didymus
Ch. furcellatus
Nitzschia seriata
Fragilaria spp.
Thalassiothrix longissima

Table 1. Relative abundance of important diatoms in each region
(In percent of cell number)

Main constituent / Region	1	2	3	4	5	6	7	8	9	10	11	12	13
Station No.	6–9, 14,15	3,4, 10,11	12,13	1	4,5	16–19	21,22 24–26	20,23	27	30–32	28,29	33,34	35–37
Chaetoceros–Phaeoceros	3	1	5	2	45	9	6	9	6	57	35	3	2
Chaetoceros–Hyalochaete	13	2	31	83	4	26	2	6	40	1	25	82	9
Denticula sp.	+	9	2	1	15	17	3	2	+	1	2	3	1
Melosira sulcata										9		+	
Nitzschia seriata	84	18	14		28	21	4	4	47	9	25	2	30
Rhizosolenia hebetata f. *semispina*		+				3	2	68	+	+	1	4	58
Rhiz. heb. f. *hiemalis*	+	1	1			4	18	+		+	8	+	
Thalassiothrix longissima	+	62	33	5	4	19	63	10	1	11	4		
Other Diatoms	+	7	14	9	4	2	2	1	6	12	+	+	

Relative abundance of these species in percentage occurring in each catch is summarized in Table 1. The hydrographic observations in the present cruise have been analyzed by Asst. Prof. H. Koto, but not yet published. Through his courtesy the isotherms and isohalines at 25 meter depth are presented in Fig. 3.

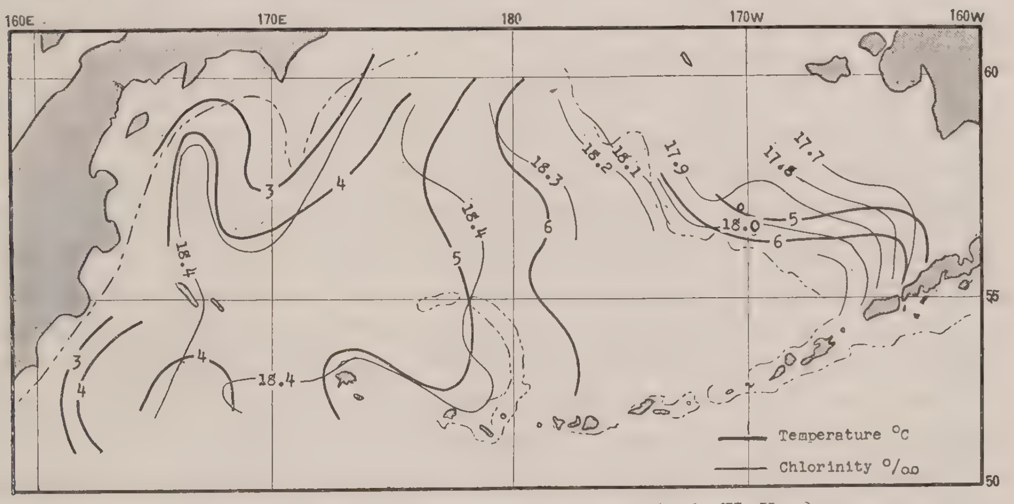


Fig. 3. Isotherms and isohalines at 25 meter depth (H. Koto)

Upon the basis of the characteristics in composition of diatom associations in upper 50 meter zone, the area investigated may be divided into thirteen regions (Fig. 4), and

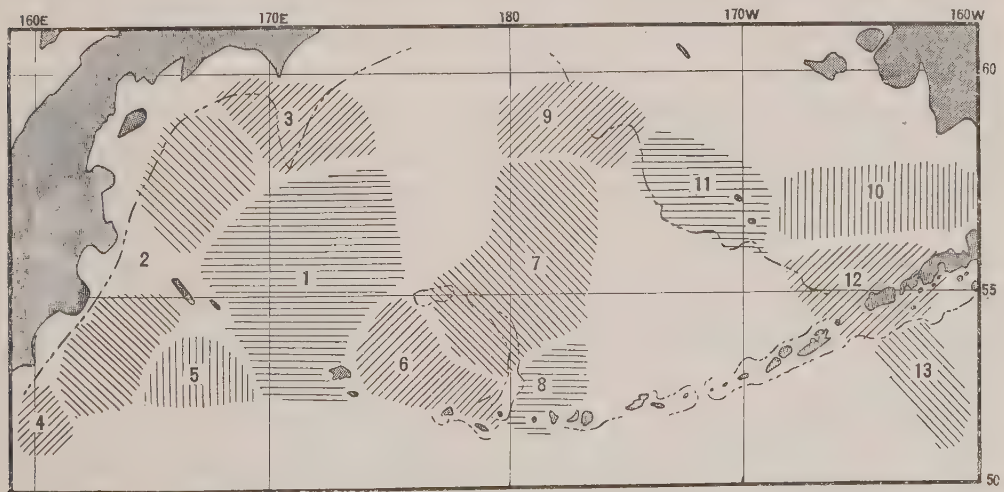


Fig. 4. Division of area according to the characteristics of diatom populations

the presentation of comments will be in order of these regions.

Region 1 (St. Os 6–Os 9, Os 14 & Os 15): In the area covering St. Os 6, Os 7 and Os 15, *Nitzschia seriata* is abundant, occupying more than three-fourths of total

diatoms, and it is followed by *Chaetoceros-Hyalochaete*, mainly composed of *Ch. debilis*, *Ch. seiracanthus*, *Ch. decipiens*, *Ch. compressus* and *Ch. teres*, altogether which are less than 20% of total diatoms. It is indicated that the population generally includes neritic elements, except in the northern part (St. Os 8, Os 9 & Os 14) where such cold oceanic forms as *Chaetoceros convolutus*, *Thalassiothrix longissima* and *Denticula* sp. are present.

Region 2 (St. Os 2, Os 3, Os 10 & Os 11): In the western Bering Sea off the east coast of Kamchatka, cold-water species such as *Thalassiothrix longissima*, *Fragilaria* spp., *Denticula* sp. and *Nitzschia seriata* are widely distributed. The first species supplies more than a half of total diatoms. The dominance of these species may indicate the prevalence of cold water that flows southward along the coast of Kamchatka (Sverdrup *et al.*, 1942).

Region 3 (St. Os 12 & Os 13): Also *Thalassiothrix longissima* is dominant. *Hyalochaete* including *Chaetoceros debilis*, *Ch. compressus*, *Ch. decipiens*, *Ch. seiracanthus* and *Ch. furcellatus* is also numerous. This subgenus may indicates neritic influence in the southern offing of Cape Olyutorskii.

Region 4 (St. Os 1): In the offing to the southeast of Kamchatka, predominance of *Chaetoceros furcellatus* is observed, as abundant as about four-fifths of total diatoms. *Chaetoceros debilis*, *Ch. atlanticus*, *Fragilaria* spp. and *Thalassiothrix longissima* are found too, but very few in number. Predominance of such neritic forms has been also reported by previous workers in the area to the south of Kamchatka (Aikawa, 1938, 1940; Motoda & Kawarada, 1955; Marumo, 1956).

Region 5 (St. Os 4 & Os 5): Differing from the neighbouring stations, St. Os 4 and Os 5 are populated by somewhat peculiar composition, i. e., subgenus *Phaeoceros* (45%) containing *Chaetoceros convolutus*, *Ch. concavicornis* and *Ch. atlanticus*, and *Nitzschia seriata* (28%). In small quantities, *Thalassiothrix longissima*, *Corethron hystrix* and *Denticula* sp. are present. These diatom species reflect the cold oceanic nature of water in this area, agreeing with the results of previous investigations (Tsuruta & Chiba, 1954; Motoda & Kawarada, 1955). It is noted that the cold oceanic flora in this region is different in composition from that off east coast of Kamchatka (Region 2).

Region 6 (St. Os 16- Os 19): Along the northern side of Aleutian Islands, from Near Islands to Rat Islands, there are mixed associations, composed of *Hyalochaete* (26%), *Phaeoceros* (9%) and other oceanic forms. They are *Chaetoceros debilis*, *Ch. seiracanthus*, *Ch. atlanticus*, *Ch. convolutus*, *Denticula* sp. (17%), *Nitzschia seriata* (21%) and *Thalassiothrix longissima* (19%), but at St. Os 17 the last two species are considerably dominant. Predominance of *Hyalochaete* was reported by Motoda & Kawarada (1955) from the waters of western Aleutian Islands, but the present data show

that considerable portions of populations in the same area are occupied by oceanic forms.

Region 7 (St. Os 21, Os 22, Os 24 – Os 26): Region 7 covers both sides of Bower's Bank and extends toward the north. *Thalassiothrix longissima* is a leading species, more than 60%, and next comes *Rhizosolenia hebetata* f. *hiemalis*. But at St. Os 26 the latter species is exceptionally numerous. *Chaetoceros atlanticus*, *Nitzschia seriata*, *Denticula* sp. and *Ch. debilis* occur in certain number at St. Os 25 and Os 26. The above dominant forms reflect the cold oceanic nature of the water.

Region 8 (St. Os 20 & Os 23): In the south of the east side of Bower's Bank, rich diatom populations are found, as many as more than 21 millions. The populations are characterized by the dominance of *Rhizosolenia hebetata* f. *semispina*, *Thalassiothrix longissima*, *Nitzschia seriata* and several species of *Hyalocheate*. It is shown that similar diatom floras are to be found between here and the south of Alaska Peninsula (St. Os 37); e. g., *Rhizosolenia hebetata* f. *semispina* is found to constitute a major portion of populations in both regions.

Region 9 (St. Os 27): This station is located close to the edge of the shallow shelf of the eastern Bering Sea. Diatoms are remarkably rich, and represented by *Nitzschia seriata* (47%) and *Hyalochaete* (40%) including *Chaetoceros debilis*, *Ch. seiracanthus*, *Ch. subsecundus*, etc. *Chaetoceros concavicornis*, *Fragilaria* spp. and *Thalassiosira decipiens* are also present in small number. These associations are generally resemblant to those in Region 1. The transparency of water measures only 6 meters, and the temperature falls from 6.7 °C at 25 meter depth to 2.4°C at 50 meter depth.

Region 10 (St. Os 30–Os 32): In Bristol Bay large portions of diatom populations are occupied by *Chaetoceros convolutus* and *Ch. concavicornis*, accompanying with *Coscinodiscus* spp., *Nitzschia seriata* and *Melosira sulcata*. Contrary to the expectation, neritic associations are not observed. General poverty of diatoms and predominance of *Phaeoceros* indicate that the central portion of Bristol Bay is much affected by cold oceanic water.

Region 11 (St. Os 28 & Os 29): This region is located on the shallow shelf of the eastern Bering Sea between Regions 9 and 10. There are present mixed associations of *Hyalochaete* (25%), mainly composed of *Chaetoceros debilis*, and *Phaeoceros* (35%), represented by *Ch. convolutus*, together with *Nitzschia seriata* (25%). Accordingly, this region may be the transitional area between Regions 9 and 10.

Region 12 (St. Os 33 & Os 34): Northwest coast of Alaska Peninsula is occupied by *Chaetoceros didymus*, *Ch. constrictus*, *Ch. debilis*, *Ch. radicans*, etc. These diatoms reflect the presence of warm water in the eastern Bering Sea. In poor number, *Rhizosolenia hebetata* f. *semispina*, *Melosira sulcata* and *Corethron hystrix* are present. It is noted that such a warm-water species as *Chaetoceros didymus* is

dominant in the Bering Sea.

Region 13 (St. Os 35 – Os 37): On the Pacific side of the Alaska Peninsula, *Nitzschia seriata* and *Rhizosolenia hebetata* f. *semispina* are prevalent, especially rich in number and dominant in composition at St. Os 37. At St. Os 35 such *Hyalochaete* species as those found in Region 12 are numerously present, giving proof of neritic influence. Toward the south, *Chaetoceros concavicornis* and *Denticula* sp. occur in certain number, evidencing an indication of oceanic influence. *Rhizosolenia hebetata* f. *semispina* is also found which has been observed as a representative diatom on the east side of Bower's Bank.

There are found some inconstancies in the distributional feature of diatoms between the results of the present observations and the previous reports. In the present cruise the greater part of the Bering Sea is found to be dominated by such forms as *Thalassiothrix longissima, Nitzschia seriata, Rhizosolenia hebetata* f. *semispina* and *Rh. heb.* f. *hiemalis*. Such wide distribution of *Nitzschia seriata* has not been reported by previous worker (Aikawa, 1936, 1938, 1940). Also the dominant occurrence of *Chaetoceros didymus* which is known as a south temperate species, in the Bering sea is noted. The distribution of *Phaeo*-plankton is fairly localized in the present cruise. *Corethron hystrix* and *Coscinodiscus* spp., the representatives of cold oceanic diatoms, are very rare compared with the previous records (Kanno, 1935; Yanagisawa, 1942; Aikawa, 1936, 1938, 1940; Marumo, 1956).

The surface diatom data by water dipping method on the same cruise as the present studies are provided by Mr. Y. Kawarada, Hakodate Marine Observatory. The diatom cell numbers are generally larger in his data than in the present. He obtained the maximum occurrence of diatoms amounting to over 10^6 cells per liter (i. e., 10^9 cells per m^3) at St. Os 27, and also found abundance of diatoms in Regions 1, 3, 12, 13 and 6. The present studies show the maximum occurrence in Region 1 amounting to over 10^7 per m^3 and also abundant occurrence in Regions 8, 9 and 13. It must be kept in mind that his data are concerned with the surface dipping, while the present data are obtained by 0-50 meter hauls.

There are general agreements in distribution of dominant forms between the above vertical and the surface data. However, there are found remarkable differences of major constituents of diatoms between the two methods of sampling in the central portions of the Bering Sea (Regions 7 and 8). In Regions 7 and 8, *Thalassiothrix longissima, Rhizosolenia hebetata* f. *semispina* and *Rh. heb.* f. *hiemalis* are dominant in the upper 50 meters while *Denticula* sp. and *Hyalochate* are exclusively dominant at the surface. There are also some inconstancies between the above two samplings in other several localities: *Hyalochaete* is exclusively dominant at the surface in Regions 6, 7 and 9, whilst mixed associatons of *Hyalochaete, Thalassiothrix longissima, Nitzschia seriata*

and *Phaeoceros* are found in the vertical haul.

In conclusion, the area investigated may be divided on the basis of certain peculiarities of diatom populations as follows: neritic areas are (1) off the east coast of Kamchatka, (2) Alaskan coast, (3) central Aleutian waters, and (4) a west portion of shallow shelf of the eastern Bering Sea. However, (1) and (2) are distinguished from (3) and (4), by the different representatives of diatom species. Oceanic areas are (5) almost all parts of the western Bering Sea and (6) portions of the eastern Bering Sea. In this demarcation, there is a disagreement between the present data and the results of observations by Johnson (1953) and Vinogradov (1956), e. g., the area (6), contrary to their cases, is indicated as an oceanic area by the present data. It has been reported that the water highly influenced by neritic elements from Alaskan coast prevails in the eastern Bering Sea and this water flows northward along the Alaskan coast (Barnes & Thompson, 1938; Goodman *et al.*, 1942; Sverdrup *et al.*, 1942). However, the diatom distribution observed on the present cruise does not confirm the above statements in the point that the eastern Bering Sea is indicated to be oceanic by the dominant presence of cold oceanic populations.

IV. Summary

Observations of the diatom populations in the Bering Sea in the summer of 1955 were made. The density of diatoms as a whole varies between 100000 and 80000000 cells per 1 m^3 of water.

Cold oceanic populations represented by *Thalassiothrix longissima*, *Rhizosolenia hebetata* f. *hiemalis*, *Fragilaria* spp. and *Denticula* sp. are widely distributed in the western Bering Sea (Regions 2 & 7) (Fig. 4). In Region 1 *Nitzschia seriata* is dominant, accompanying with subgenus *Hyalochaete*, which represents somewhat neritic nature.

In the eastern Bering Sea and southwestern part of the western Bering Sea an oceanic group, *Phaeoceros*, is distributed in certain restricted locations (Regions 5 & 10).

Neritic populations represented by *Hyalochaete* occur to the southeast of Kamchatka, in western Bering Sea and to the east of the shallow shelf of the eastern Bering Sea (Regions 4, 12 & 9).

Mixed associations of subgenus *Hyalochaete* and other oceanic forms are found in Aleutian waters (St. Os 16 – Os 19), to the south of Cape Olyutorskii and in the eastern Bering sea, suggesting the mixing of oceanic and neritic waters (Regions 3,6 & 11).

Rhizosolenia hebetata f. *semispina* and *Nitzschia seriata* are abundantly distributed in the offshore area of Pacific side of Alaska Peninsula, and the former species is also found in the east of Bower's Bank (Region 8 & 13).

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REPORT FROM THE "OSHORO MARU" ON OCEANOGRAPHIC AND BIOLOGICAL INVESTIGATIONS IN THE BERING SEA AND NORTHERN NORTH PACIFIC IN THE SUMMER OF 1955

V. Observations on Copepod Community [1)]

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Introduction

Participating in the International Cooperative North Pacific Oceanographic Programme held in the summer of 1955 the training ship "Oshoro Maru" of the Faculty of Fisheries, Hokkaido University, made a cruise to the Bering Sea and northern portion of the North Pacific (Motoda & Fujii, 1956). Prof. S. Motoda was aboard the ship and conducted the research work at sea in this cruise. He placed a part of the plankton materials obtained in this cruise at the disposal of the author. The present studies are undertaken to observe the distribution figure of copepods which might be represented by gross pattern of hydrography.

The collection of zooplankton was made regularly at hydrographic stations by vertical hauls, usually from 150 metre depth to the surface with 45×165 cm net (0.33 mm mesh aperture); hauls were duplicated. A deep haul with 63×300 cm net (0.49 mm mesh aperture) was made at one station (Os 8) by making separate hauls with closing

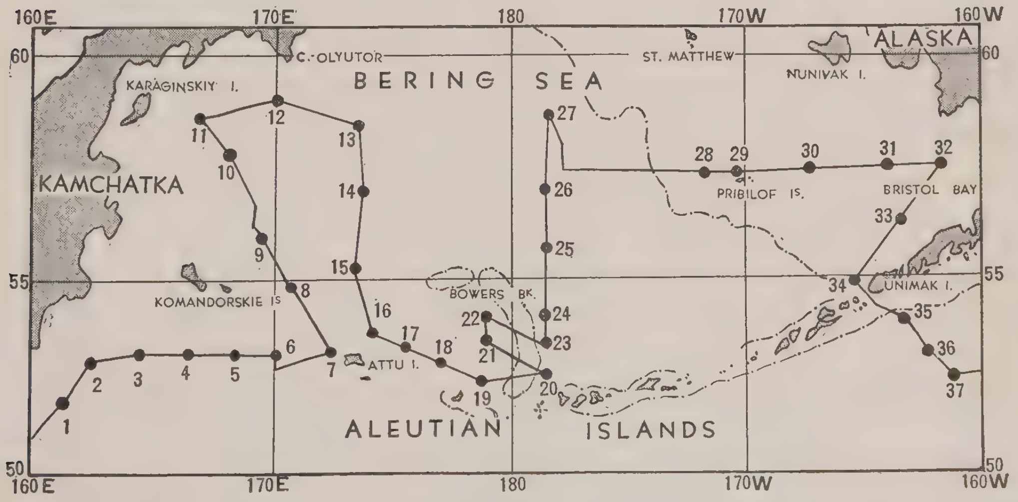


Fig. 1. Approximate location of sampling stations

1) おしょろ丸北洋調査報告 No. 9 (昭和30年度)
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mechanism from 2000 to 1000 metres, from 1000 to 500 metres and 500 to the surface. In addition, deep hauls from usually 1000 metre depth with a couple of rectangular nets, "Twin Net" (10×10 cm at mouth, 50 cm long, 0.28 mm mesh aperture), were performed at every station. Detailed descriptions of the gear and methods of collections and of the exact location of stations are presented in the paper of Motoda and Fujii (1956); only a sketch map showing approximate location of stations is given here (fig. 1).

On this occasion the author wishes to express his sincere gratitude to Prof. S. Motoda not only for his generosity in providing the materials to the author but for his guidance throughout the present studies. He also expresses cordial thanks to Asst. Prof. T. Kawamura and Mr. M. Anraku for their valuable advices during the present studies. The writer is also obliged to the research members and crew of the "Oshoro Maru" in 1955 cruise for their work in sampling the materials at sea.

Results of Observations

The data on quantitative estimation on regular collections with 45×165 cm net are presented in "*Data Record of Oceanographic Observations and Exploratory Fishing*, No. 1, 1957". These data are concerned with wet displacement volume and wet weight of the materials, but in the present studies individual numbers of copepods are counted for each species (tables 1 & 2). As the net was not equipped with a flow-meter, exact filtration coefficient of the net was unknown, but the volume data were converted by assuming that the filtration coefficient of such net would be 0.76 (Motoda *et al.*, 1957). In addition, the error of hauling distance which may have happened in rough weather was corrected (Motoda *et al.*, 1957).

1) Copepod species identified

The copepod species identified from the materials of regular collections with 45×165 cm net are as follows:

* *Calanus finmarchicus* (GUNNERUS)
* *C. plumchrus* MARUKAWA
* *C. cristatus* KRÖYER
* *Eucalanus bungii bungii* JOHNSON
* *Pseudocalanus elongatus* (BOECK)
Microcalanus sp.
Aetideus armatus (BOECK)
Gaetanus armiger GIESBRECHT
* *Gaidius tenuispinus* (SARS)
* *Pareuchaeta japonica* (MARUKAWA)
** *Racovitzanus antarcticus* GIESBRECHT
* *Scolecithricella minor* (BRADY)

*** *Centropages abdominalis* SATO
* *Metridia lucens* BOECK
** *Pleuromamma scutullata* BRODSKY
** *Lucicutia ovaliformis* BRODSKY
* *Candacia columbiae* CAMPBELL
*** *Acartia longiremis* (LILLJEBORG)
*** *Oithona plumifera* BAIRD
*** *O. similis* CLAUS
*** *Microsetella norvegica* (BOECK)
Oncaea conifera GIESBRECHT

A deep haul with 60×300 cm net yielded 27 species, of which the follwoing 11 species are never found in the above regular collections from the upper zone.

* *Microcalanus pygmaeus* (SARS)
** *Spinocalanus spinipes* BRODSKY
** *Gaidius brevispinus* (SARS)
* *Pareuchaeta birostrata* BRODSKY
** *Scaphocalanus magnus* (T. SCOTT)
** *Onchocalanus affinis* WITH
** *Amallothrix inornata* (ESTERLY)
* *Scolecithricella ovata* (ESTERLY)
** *Metridia asymmetrica* BRODSKY
** *Heterorhabdus tanneri* (GIESBRECHT)
* *Oncaea notopus* GIESBRECHT

In addition, deep hauls with a small "Twin Net" yielded the following additions to the above two kinds of collections:

** *Spinocalanus abyssalis* GIESBRECHT
Scaphocalanus sp.
** *Pachyptilus pacificus* JOHNSON
** *Arietellus simplex* SARS

The majority of the above-mentioned species are those which have been reported to be either the boreal (*) or abyssal (**) forms, but a few are forms (***) whose distribution is known to be neritic and world-wide.

Brief mention will be made here on several species whose names have been somewhat confused or whose distribution is especially interesting.

Calanus plumchrus MARUKAWA was first described by Marukawa (1921) as new to science, but the first record in Japanese waters was earlier published by Sato (1913) under the name of *Calanus* sp. They both observed only the immature specimens. The adult forms were found from the deep water (Yamada, 1938; Nakai, 1942; Motoda *et*

al., 1950; Anraku, 1954a, b). Brodsky (1938; 1948; 1950) stated that this species is synonymous to *Calanus tonsus* and he divided it to two forms, *plumchrus* and *typica*. Tanaka maintained the same opinion in his past paper (1954), but after that he (1956) proposed that *Calanus plumchrus* is distinct from *Calanus tonsus*.

According to Johnson (1939), *Eucalanus bungii bungii* JOHNSON is a northern variety of *Eucalanus bungii*, and a southern variety is *Eucalanus bungii californicus*. He stated that the former variety is distributed from the northern area of Japanese waters to the Bering Sea and the latter along the California coast, but var. *californicus* was reported by Tanaka (1953) in the deep water of Sagami Bay.

Pareuchaeta japonica (MARUKAWA) was originally described by Marukawa (1921) as *Euchaeta japonica*. Brodsky (1948; 1950) reported this species from the Japan Sea, Okhotsk Sea, Bering Sea and northern North Pacific. He followed the classification of A. Scott (1909) who divided family Euchaetidae into two genera, *Euchaeta* and *Pareuchaeta*. Mori (1937) stated that the variation between the two genera is obscure and gradual, and Davis (1949) had the same opinion.

Centropages abdominalis SATO was first reported by Sato (1913) near Hokkaido, Kii Channel and Terpeniye Bay of Sakhalin. Later this species was treated as a synonym of *Centropages mcmurrichi* WILLEY 1920 by Brodsky (1948; 1950). However, the publication of Sato (1913) was the earlier, so that *Centropages abdominalis* should be used. This species is distributed in the inland sea of Japan, Okhotsk and Bering Sea, Yellow Sea and southern area of Chukchee Sea (Brodsky, 1950), Arctic Ocean, northern area of Bering Straits, Bering Sea, Grantley Harbour, Alaska, Dixon Entrance and the area adjacent to Vancouver Island (Davis, 1949).

Spinocalanus spinipes, *Pareuchaeta birostrata*, *Metridia asymmetrica*, *Pleuromamma scutullata* and *Lucicutia ovaliformis* were reported by Brodsky (1950) from the northern North Pacific, Bering Sea and Okhotsk Sea. These species are oceanic, bathybic or abyssal.

2) Characteristic distribution of representative copepod species

It is reported that the water which originated from the Gulf of Alaska flows westward and turns northward into the Bering Sea passing through the Aleutian Chain. Some of this water passes out through the Bering Straits and some is flowing as a counterclockwise circulation in the Bering Sea. Along the eastern coast of Kamchatka Peninsula, a part of this current flows still farther southward and finally it is mixed with the water which has poured from the Okhotsk Sea, while another part of the water is thrust into the Subarctic Water and reached the Gulf of Alaska (Fleming, 1955; Mishima & Nishizawa, 1955).

Among the copepods colleted, *Calanus finmarchicus*, *C. plumchrus*, *C. cristatus*,

Eucalanus bungi bungii, *Pseudocalanus elongatus*, *Metridia lucens*, *Acartia longiremis* and *Oithona similis* are numerically important.

All specimens of *Calanus plumchrus* in the samples of 45×165 cm net represent copepodid stages I to V, while the adult forms were usually collected by deep hauls with "Twin Net" and with 63 × 300 cm net. Off the east of Karaginskiy Island (Os 11), and off the southeast of Cape Olyutor (Os 13) and at Os 15, this species occupied the bulk of samples counting more than 200000 individuals per 1000 m³ of water, while in other stations (Os 2, 8–10, 12, 14, 16, 17, 21, 22, 24 and 26) the number of individuals was 100000 or more. At Os 4 and 5 off the southern part of Komandorskie Islands and at Os 28 and 29 in the shallow eastern Bering Sea, this species occurred to the number of about 5000 individuals per 1000 m³ of water. In shallow hauls on the continental shelf extending from Alaska (Os 28–30, 33 and 34) this species diminished gradually, but the adult forms were collected there in spite of shallow hauls, probably being brought in with water upwelling from the deep. No specimen of this species was collected at Os 31 and 32 in Bristol Bay.

Calanus finmarchicus appeared in considerable number replacing *C. plumchrus* in Bristol Bay. The number was counted as 3000000 individuals per 1000 m³ of water in one of the duplicate hauls at Os 31. In the west and central Bering Sea except neighbouring Bower's Bank this species disappeared in the samples of 150 metre vertical hauls as well as deep hauls.

Immature individuals of *Calanus cristatus* occupied the largest bulk of 45×165 cm net samples at Os 11 and 27, counting about 5000 to 9000 individuals per 1000 m³ of water. The number of individuals was between 100 and 5000 in the west and central Bering sea. In Bristol Bay (Os 30, 31 and 32) this species was not collected. The adult forms were sometimes collected only from the deep layer below 150 metre depth in the west and central Bering Sea.

Eucalanus bungii bungii occurred in extraordinary abundance at Os 16, 17 and 18, about 1500000 individuals per 1000 m³ of water, and at Os 11 and areas near Bower's Bank (Os 20, 22 to 26) the number was also very large. But no one was collected at Os 30 to 32. The adult forms were included in the samples of 150 metre vertical hauls at every station.

Pseudocalanus elongatus was widely distributed at all stations, the most numerous in Bristol Bay (Os 30 to 32), but comparatively less in number in the off-shore region of the Bering Sea and south of Alaska Peninsula.

Very abundant copepodids of *Metridia lucens* with a few adult forms were collected around Bower's Bank (Os 20 to 26) in 150 metre hauls. At Os 11 only copepodid stages were collected rather abundantly in 150 metre hauls, while many adult forms appeared in the deep "Twin Net" samples. This species become less in the southwestern

Bering Sea, *i. e.*, area south of Komandorskie Islands (Os 2 to 6), at Os 30 and 33. Neither copepodid nor adult occurred at Os 31 and 32.

Oithona similis occurred in the largest number off the eastern coast of Kamchatka (Os 2 and 11) in the 150 metre hauls, and diminished gradually to the offing. In the neighbourhoods of Near Islands, Bower's Bank and Bristol Bay except Os 32, it was comparatively increased in number.

Acartia longiremis was collected near Bower's Bank, Bristol Bay and Alaska Peninsula in 150 metre vertical hauls.

Scolecithricella minor was collected by 150 metre hauls in all stations except Os 29 to 32 in Bristol Bay.

Centropages abdominalis was collected at Os 1 off the east of Kamchatka and Os 33 to Os 36 near Unimak Pass.

Among copepods collected by the deep hauls with "Twin Net" and with 63 × 300 cm net, *Gaetanus armiger*, *Racovitzanus antarcticus*, *Pleuromamma scutullata* and *Lucicutia ovaliformis* were also sometimes collected by 150 metre vertical hauls. From upper 150 metre zone, *Gaetanus armiger* was collected near Bower's Bank (Os 20 and 21) and in the southern portion of Unimak Pass (Os 36), again *Racovitzanus antarcticus* in the west and central Bering Sea, and the southern portion of Unimak Pass, *Pleuromamma scutullata* at Os 10, 14, 20 to 22 and 35, and *Lucicutia ovaliformis*

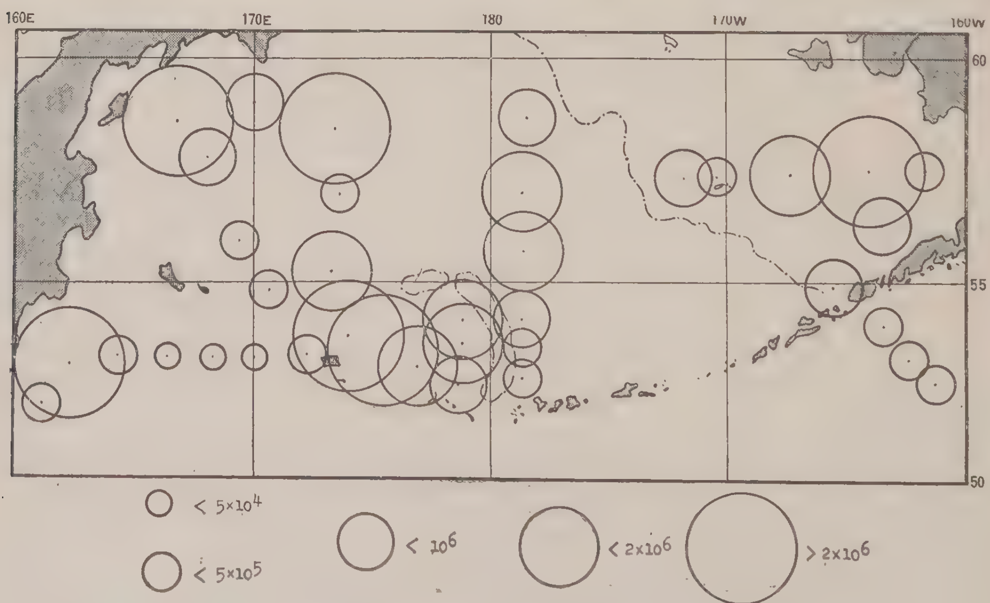


Fig. 2. Number of copepods occurring in upper 150 metre zone (Individual numbers/1000 m³ of water)

at Os 9.

In the upper 150 metre zone, *Calanus plumchrus*, *C. cristatus*, *Eucalanus bungii bungii*, *Metridia lucens* and *Oithona similis* were distributed normally and abundantly in the Bering Sea except Bristol Bay. They were as a whole the most abundant at Os 2, 11 and 13 off the east coast of Kamchatka and Os 16 and 17 off the northeast of Attu Island; comparatively abundant in the northern and western portions of Bower's Bank (Os 15, 18, 21, 22, 25 and 26) (fig. 2).

In Bristol Bay, the community of copepods, comprising mainly *Calanus finmarchicus*, *Pseudocalanus elongatus* and *Acartia longiremis*, was the most numerous at Os 31, with Os 30 following (fig. 2).

In the south of Unimak Pass, *Calanus plumchrus*, *C. cristatus*, *Eucalanus bungii bungii* and *Metridia lucens* increased again, while *Calanus finmarchicus* decreased.

Discussion

In the present observation, so far as the upper zone (0–150 metres) is concerned, two divisions of distributional area of copepods are considered, that is, *Calanus plumchrus*—*Eucalanus bungii* area in the west and east Bering Sea, and *Calanus finmarchicus*—*Acartia longiremis* area in the eastern shallow Bering Sea (fig. 3).

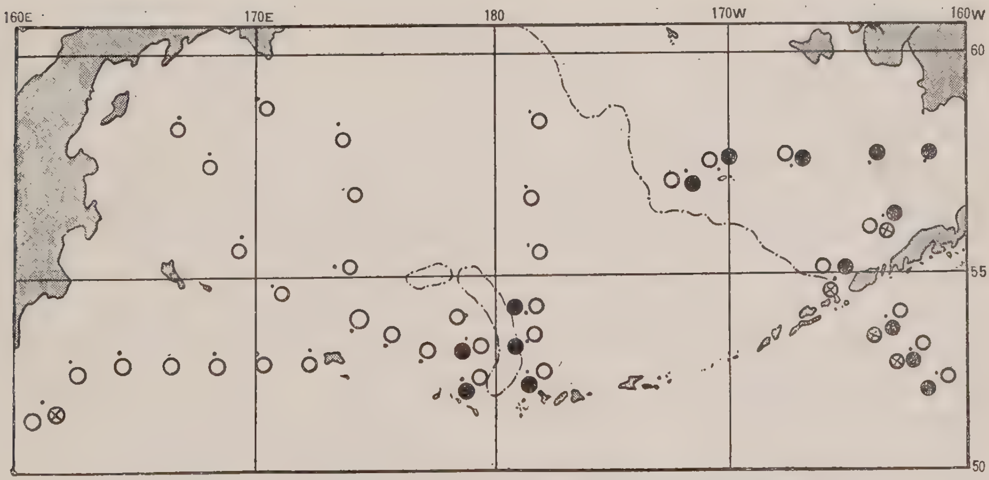


Fig. 3. Distribution figure of three types of copepod populations
○ *Calanus plumchrus—Eucalanus bungii* population
● *Calanus finmarchicus—Acartia longiremis* population
⊗ *Centropages abdominalis*

Calanus plumchrus—*Eucalanus bungii* population nearly all consisting of copepodids and nauplii usually occurs in company with *Calanus cristatus* and *Metridia*

lucens. They are all oceanic forms. *Oithona similis*, eurythermic and euryhaline copepod form, is also included in these populations. The above population involving these species is widely distributed in the west and central Bering Sea making up the largest group off the east coast of Kamchatka and in western portion of Bower's Bank, but they are rare in the western area of the Near Islands. They are diminishing toward the inshore of Alaska and replaced by *Calanus finmarchicus—Acartia longiremis* population.

In the region to the west of the Pribilof Islands and adjacent to Unimak Pass a mixed population of *Calanus plumchrus—Eucalanus bungii* and *Calanus finmarchicus—Acartia longiremis* is observed.

Calanus finmarchicus—Acartia longiremis population is found only at Os 31 and 32 in Bristol Bay.

Johnson (1953) reported two communities in the eastern Bering Sea and in the Chukchee Sea; (1) a western community characterized by offshore deep-water species extends somewhat eastward just north of the Aleutian Islands, and northward through the Bering Straits on the Siberian side, (2) an eastern neritic community characterized by neritic or estuarine forms exists in the Pribilofs, Nunivak Island and off the Alaskan coast. According to Brodsky (1955), the population in the offshore of the southern Bering Sea is similar to the northern North Pacific population consisting of *Calanus tonsus*, *C. cristatus*, *Eucalanus bungii*, *Scolecithricella minor*, *Pareuchaeta japonica* and *Metridia pacifica*, extending to the Gulf of Alaska, to near southeastern Hokkaido and to the central Okhotsk Sea. The neritic population of eastern Bering Sea is extending along the Aleutian Chain to Near Islands. Vinogradov (1956) reported that the copepod population in the offshore of southern Bering Sea extends far to the Bering Straits and the population of the coastal region of west Bering Sea occurring abundantly in the Gulf of Anadir is extending far to the region adjacent to the Pribilof Islands; moreover, *Calanus tonsus* and *Eucalanus bungii* constitute characteristic southern population, and *Calanus finmarchicus* occurs in the population in the Gulf of Anadir.

In the area adjacent to Bower's Bank, copepods collected are only oceanic and bathybic forms (Anraku, 1954b), while diatom population is occupied by neritic forms (Motoda & Kawarada, 1955).

In the present cruise, it is considered that the water from the Pacific pouring into the Bering Sea is characterized by *Calanus plumchrus—Eucalanus bungii* population which are oceanic and bathybic forms, and the water in the eastern shallow Bering Sea is characterized by presence of *Calanus finmarchicus—Acartia longiremis* population which are boreal or neritic forms. The water in Bower's Bank, around the Pribilof Islands and in Unimak Pass are inhabited by the mixed populations of the above forms.

Summary

Regular samplings were made by 150 metre vertical hauls with 45 × 165 cm net and by 1000 metres or more deep hauls with a couple of 100 cm^2 × 50 cm "Twin Net". A deep haul extending to 2000 metres was made with 63 × 300 cm net at one station. Thirty-seven species of copepods were identified from such collections.

So far as the upper 150 metre zone is concerned, *Calanus plumchrus*, *C. cristatus*, *Eucalanus bungii bungii*, *Scolecithricella minor* and *Metridia lucens* which are oceanic forms, are commonly distributed in the west and central Bering Sea, and in the southern offshore region of the Alaska Peninsula. *Calanus finmarchicus* and *Acartia longiremis* are distributed around Bower's Bank and in the eastern shallow Bering Sea. *Pseudocalanus elongatus* and *Oithona similis* are widely distributed in the Bering Sea and in the southern offshore region of the Alaska Peninsula. The distribution of *Centropages abdominalis* is restricted to the southern side of Kamchatka and waters adjacent to Unimak Pass.

In the upper zone of the Bering Sea, three areas are distinguished with respect to the copepod populations; that is, (1) *Calanus plumchrus*—*Eucalanus bungii* area in the west and central Bering Sea, (2) *Calanus finmarchicus*—*Acartia longiremis* area in the eastern shallow Bering Sea, (3) Mixed area of these forms around Bower's Bank, the Pribilof Islands and Unimak Pass.

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Table 2. Number of individuals (per 1000 m^3 of water) of each species of copepoda occurring in separate vertical zone at station Os 8
(Error of filtration coefficient of the net is not corrected)

Species	500–0m	1000–500m	2000–1000m
Calanus plumchrus (adult)	928	13	13
〃 (copepodid)	3377	96	22
C. cristatus (adult)	19	58	10
〃 (copepodid)	600	232	
Eucalanus bungii bungii (adult)	2858	426	3
〃 (copepodid)	1109	84	6
Pseudocalanus elongatus (adult)	671	26	3
〃 (copepodid)	103	45	
Microcalanus pygmaeus		19	16
Spinocalanus spinipes	52	19	
Gaidius tenuispinus	52	45	13
G. brevispinus	103	71	10
Gaidins 〃 copepodid	103		
Gaetanus armiger	180	32	3
Pareuchaeta japonica	19		3
P. birostrata	32	13	3
Pareuchaeta 〃 copepodid	491	129	6
Scaphocalanus magnus	52	6	3
Onchocalanus affinis		13	
Amallothrix inornata	103	45	
Racovitzanus antarcticus	129		
Scolecithricella minor	516	32	6
S. ovata		19	
Scolecithricella 〃 copepodid	77	142	
Metridia lucens	129	174	3
M. asymmetrica		206	16
Metridia 〃 copepodid	2476	123	6
Pleuromamma scutullata	439	13	
Lucicutia ovalifromis	181	497	23
Heterorhabdus tanneri	52	581	
Oithona similis	491	194	61
O. plumifera		6	
Microsetella norvegica	26		
Oncaea conifera	491	155	6
O. notopus			10
Other copepodid stages	774	271	16
Copepod nauplii	155	45	6
Total	16788	3930	267

BLOOD CELL CONSTITUENTS IN FISH

II. Peroxidase Staining of the Leucocytes in the Kidney and Some Other Organs

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In the previous paper (Yûki, 1957), the author reported that the leucocytes of the blood stream in the teleost fishes clearly show the positive reaction by the peroxidase staining, that these stained cells are easily discernible from the other blood elements owing to their characteristic colouration, and that fluctuation of the ratio measured between the positive and negative cells is utilizable in fish physiology as a biological indicator.

In course of the present study, it was found that the positive leucocytes in the blood stream fluctuate in number markedly corresponding to some unknown physiological changes in the fish. For example, sometimes these cells increase to several times the usual number, while in other cases they decrease to as few as almost none, though those in the blood stream are usually small in number. It is presumable that such a fluctuation of the positive cells is a response of the hematopoietic organ to physiological changes of the fish. To make clear the causes of such a fluctuation in the number of these cells, examination was first made in which organ they are generated and stored abundantly.

The author owes thanks to Prof. Shinjirô Kobayashi, for his constant guidance and suggestions rendered during the course of this work, and for his kindness in the revision of the manuscript. Thanks are also offered to Assist. Prof. S. Hamano, who gave invaluable advice during this work. The author is also indebted to Assist. Prof. T. Kubo, who aided in supplying the materials used.

MATERIALS AND METHODS

In the present experiment, the rainbow trout, *Salmo irideus*, 2 – 4 years old was mainly used, which had been reared at Nanae, near Hakodate City. Further, the following forms and species of fishes were also examined: adult marine and young fresh-water forms of Masu, *Oncorhynchus masou*, anadromous form of chum salmon, *O. keta*, Atka mackerel, *Pleurogrammus azonus*, 2 species of rockfish, *Sebastes taczanowskii* and *S. thompsoni*, 2 species of puffer, *Fugu vermicularis porphyreus* and *F. vermicularis paradalis*, starry flounder, *Platichthys stellatus*, and wingfish, *Lepidotrigla microptera*. All these fishes were collected in the natural field, river and sea.

The peroxidase-positive leucocytes in the following organs were examined: liver, kidney (mesonephros), spleen, gill, pyloric caeca and brain. Before dissection of the

fish is made, enough blood was drained out cutting the caudal part. From the blood thus obtained, the blood serum was prepared for use in the following mince- and smear-preparation of the organs. A piece of each organ taken from the fish is set on a slide glass, and a few drops of the serum are poured on it. Then, the piece is minced with scissors, and the smear preparation of the organ is made. Finally, the residues of the piece are removed from the slide glass. The smear preparations thus made were stained by the two methods, Wright-Giemsa's and peroxidase stainings the same as used in the previous observation (Yûki, 1957).

OBSERVATIONS

The peroxidase-positive leucocytes were found in most of the organs examined, and they were identical with those which were observed in the blood stream. Among the organs examined, the positive cells were found most abundantly in the kidney. Further, as shown in Fig. 2, a, the other elements of the blood cells belonging to the various stages were also observed abundantly in this organ.

In the case of the mince- and smear-preparations employed in the present observation, it is difficult to indicate the absolute number of the positive cells occurring in each organ as they are not smeared in most cases so as to distribute uniformly on a slide glass. However, it seems reasonable that an approximate tendency of the occurrence of these cells in the organ may be judged by the following method. That is, in place of a micrometer, a glass plate with a notched area of 4×6mm is inserted into the ocular of the microscope; sliding the preparation from side to side, sums of the respective numbers of the erythrocytes and positive cells observed successively within the notched area are counted under the microscope; then the ratio of these two kinds of blood cells is calculated. The following table shows the number of the positive cells per 10,000 erythrocytes in each organ and that of the blood stream in the rainbow trout. Each number was calculated from the average of 3 measurements on each preparation, using the method described above.

Kidney	*Spleen*	*Liver*	*Brain*	*Pyloric caeca*	*Gill*	*Blood*
4500–6100	250–400	>50	0?	0?	+?	20–30

Although as stated in the above the numbers of positive cells shown in the table do not indicate the absolute value of each organ, it is clear that the cells are much more abundant in the kidney than in the blood stream (Figs. 1 & 2). Possibly, the number of the cells in the former organ may run up to a few hundred times as many as that of the latter. In comparison with the kidney, the positive cells are much less in both spleen and liver in the rainbow trout (Figs. 3 & 4). In both brain and pyloric caeca, not a single cell which showed the positive reaction was observed, at least in the present experiment. The positive cells were found in the gill rarely, but they might possibly be

derived from the circulating blood in this organ.

Except the starry flounder and wingfish, the positive leucocytes were found to be stored abundantly in the kidney of all the forms and species of the fishes examined, similarly as in the rainbow trout. In the exceptional two species, the positive cells were found to be rather more abundant in the liver in the starry flounder and in the spleen in the wingfish than in the kidney of the respective species.

DISCUSSION

Jordan and Speidel (1924) have reported from their detailed observations concerning hematopoiesis of the fish that in teleost the intertubular connective tissue of kidney (mesonephros) is a predominant hematopoietic site and spleen is only of a secondary importance in this function. Dawson (1935) has also reported that in the teleost fishes the mesonephros is an important hematopoietic locus for both erythrocyte and granulocyte. Walter (1955) has pointed out from his recent study that the kidney is the most important organ for the hematopoiesis in carp, too. Although the method employed in the present observation differs from those of the previous workers who used either the anatomical smear or the section of tissues, the author's result also agrees with those of them.

As stated in the previous paper (Yûki, 1957), the peroxidase-positive leucocytes are composed of both groups of the monocytic and granulocytic cells. It is clearly seen in the present mince- and smear-preparations that the positive cells found in the kidney are formed also of both the monocyte and granulocyte or either of them as they included elements of various stages from the undifferentiated to the mature cells. However, it is apparently curious that these positive cells are few in the blood stream, while they are found to be abundant in the kidney. Regarding this phenomenon, the author has held the opinion that it is resulted from the following two causes. It is conceivable as the first cause that the positive cells are short-lived in the blood stream. If that is the case, abundant cells must be generated and stored in some organ to supply them continuously into the blood stream, owing to their short longevity. As the second cause, it may be considered that the abundant existence or the storage of the positive cells in the fish kidney is essential for the performance of their physiological function, besides the phagocytosis of them (Irie, 1932). Further, various kinds of enzymes which are contained within the stored positive cells are considered to have some important roles to accomplish the function of the kidney. The fact that these cells show the positive reaction to the peroxidase staining suggest that they contain the peroxidase and some other enzymes, even if the staining is only a secondary reaction as Lison (1953) has stated. In such a meaning, the present finding might throw light upon an analysis of the physiological function of the fish kidney, though it has not yet been clarified in many respects.

SUMMARY

1. To find in which organ the peroxidase-positive leucocytes are generated and stored, the mince- and smear-preparations of the liver, kidney, spleen, brain, gill and pyloric caeca were examined using both stainings of the Wright-Giemsa and the peroxidase. Although the observation was made mainly on the organs of the rainbow trout, the following forms and species of fishes were also used: adult marine and young fresh-water forms of Masu, anadromous form of chum salmon, Atka mackerel, 2 species of rockfish, 2 species of puffer, and wingfish.

2. The positive cells were found most abundantly in the kidney. In the rainbow trout, the approximate number of these cells in the kidney reaches about 200 times as many as those found in the blood stream. Probable causes concerning such a marked difference of the occurrence of the positive cells between the kidney and blood stream were discussed briefly.

3. In the rainbow trout, the positive cells of the spleen are relatively small in number being only about 1/20 that of the kidney; those of the liver are about 1/100 of the same organ. None of the positive cells were found in either brain or pyloric caeca. In the gill, they were found rarely.

4. Among the fishes examined, the occurrence of the positive cells was somewhat characteristic in the starry flounder and wingfish. That is, they are relatively abundant in the liver in the former species and in the spleen in the latter one. The results in the other forms and species of the fishes examined are almost identical with those of the rainbow trout.

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PLATE I

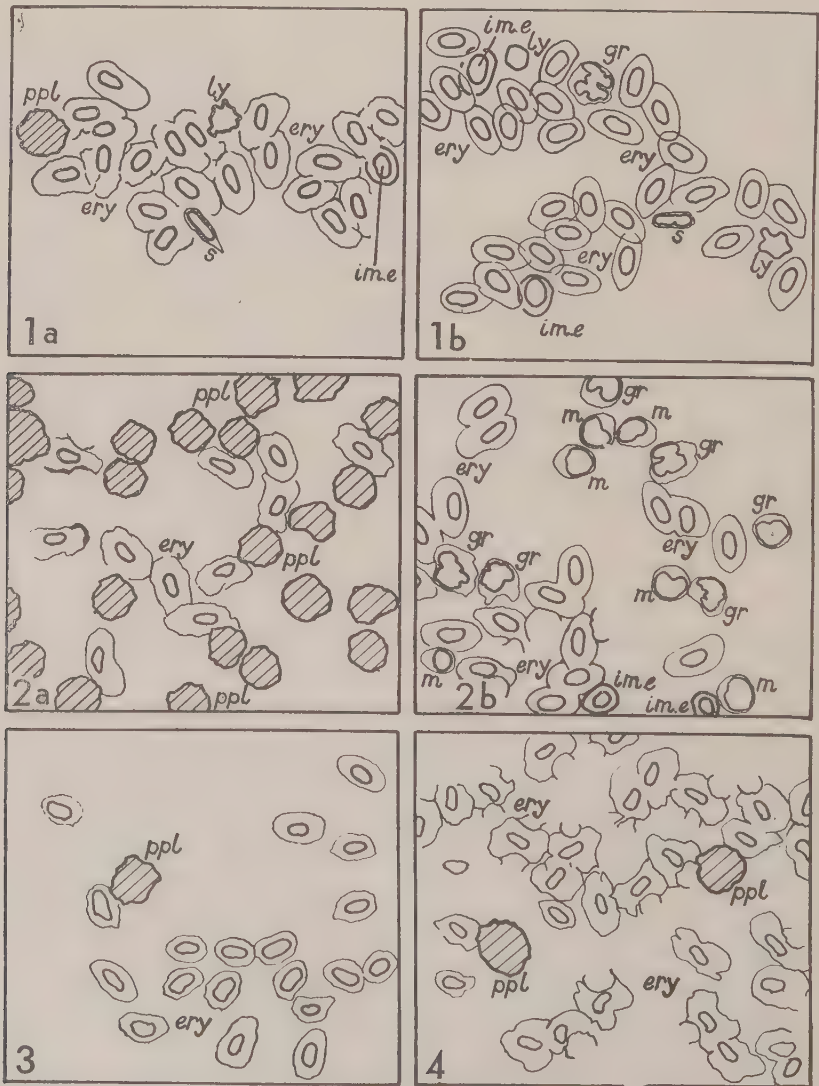
Explanation of figures

All figures were photomicrographed from the preparations of the circulating blood and certain organs in the rainbow trout. Magnification of the figures is about 600 diameters.

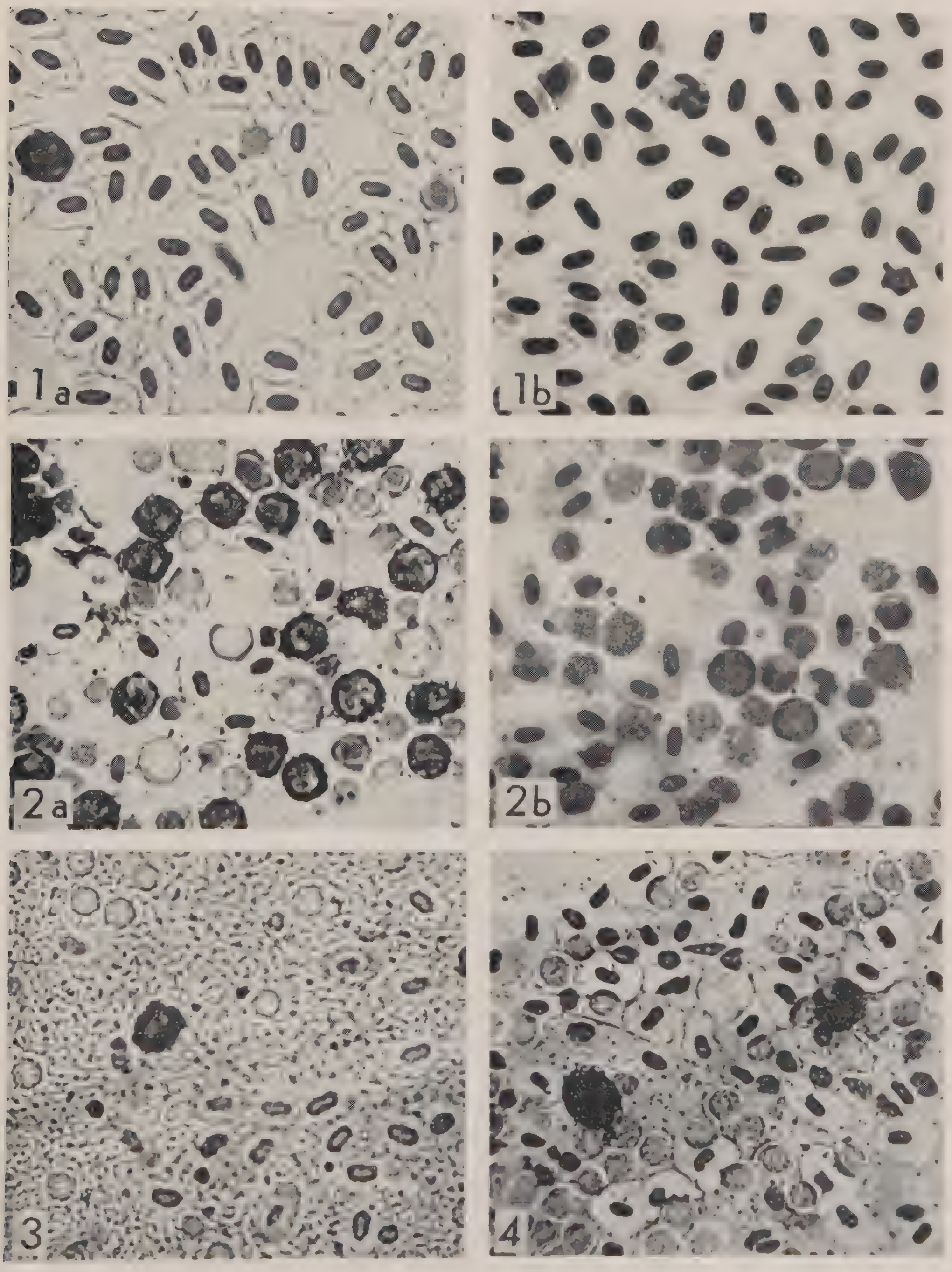
Fig. 1. Circulating-blood smears. a: peroxidase staining; b: Wright-Giemsa's staining.

Fig. 2. Mince- and smear-preparations of kidney. a: peroxidase staining; b: Wright-Giemsa's staining.

Figs. 3 and 4 show mince- and smear-preparations of liver and spleen obtained by peroxidase staining.



ery Erythrocyte *gr* Granulocyte *im.e* Immature erythrocyte *ly* Lymphocyte
m Monocyte *ppl* Peroxidase-positive leucocyte *s* Spindle cell



R. YÛKI: Leucocytes in the Kidney

STUDIES ON THE FORMATION OF FISH EGGS

XII. On the Non-massed Yolk in the Egg of the Herring, *Clupea pallasii*

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According to the form of the yolk, one can classify fish eggs into two different types, massed-yolk eggs and non-massed-yolk ones. Of these two types, the former has already been studied in the previous work, resulting in the conclusion that a continuous mass of yolk in the flounder is derived from yolk globules alone and the lipids in the globules consist mainly of phospholipids (K. Yamamoto 1957).

As for the formation of non-massed yolk, on the other hand, there can be found several valuable papers, such as those of Lams (1903) on *Osmerus*, of Konopacka (1935) on *Cyprinus* and *Gobio*, and of Osanai (1956) on *Lefua*; for the chemical nature of non-massed yolk there are the papers of Konopacka (1935) and Mas (1952). In spite of these works, there still remain many controversial points requiring elucidation. The present writer has studied some of those points, using the oocyte of the herring as the material, and obtained some findings worthy to be noted here.

Before proceeding further, the writer would like to express here his cordial thanks to Professor Tohru Uchida, Director of the Akkeshi Marine Biological Station, under whose guidance the main part of this work has been performed. The writer is also indebted to Professor Sajiro Makino, Faculty of Science, and Professor Hisanao Igarashi, Faculty of Fisheries, for their valuable advices, and also to staff members of the Akkeshi Marine Biological Station for their kindly aid extended in collecting the material.

MATERIAL AND METHOD

The oocytes of herring, *Clupea pallasii*, were obtained in a similar way to that described in the former paper (K. Yamamoto 1955a).

The lipids occurring in the yolk globules were demonstrated by Ciaccio's test and the modified Sudan black B and alcohol method. Moreover, the chemical nature of lipids was investigated by Baker's test for phospholipids, Schultze's test for cholesterol and Smith's Nile blue staining for the differentiation of phospholipids from glycerides.

Details concerning the practice of these cytochemical techniques may be learned by consulting the previous paper (K. Yamamoto 1957).

RESULTS

1. Formation of non-massed yolk globules

In herring oocytes, yolk vesicles are formed as primary vitelline particles. At the beginning of their formation, the vesicles are minute and spherical, and found scattered

in the ooplasm. They gave always a negative Ciaccio's reaction and positive P. A. S. reaction (K. Yamamoto 1955a). The vesicles then increase rapidly in size and number, and are found arranged in the periphery of the ooplasm, roughly forming a row. At the same time, very minute granules showing a strong Ciaccio's reaction are seen in the extravesicular cytoplasm. Being flocked together near the periphery of the ooplasm, the granules form a narrow ring situated outside the vesicle layer (Fig. 1). As the oocyte grows further, the formation of the vesicles proceeds by and by, to occupy a large part of the ooplasm. During this period, the Ciaccio-positive granules also increase in size and number, though not markedly (Fig. 2). At the next stage, the Ciaccio-positive granules grow rapidly in size and develop into so-called yolk globules. The yolk globules showing a strong and homogeneous Ciaccio's reaction appear at first in the peripheral cytoplasm and then invade the interstices between the yolk vesicles, and finally they fill the inner cytoplasm surrounding the nucleus. In the meanwhile, the vesicles show no marked changes, and still remain sudanophobic (Figs. 3 and 4). Then, the globules continue to grow in size and number; this activity appears to be more vigorous in the inner part of the ooplasm than in the outer part. Consequently, the vesicles seem to be shifted towards the periphery of the ooplasm, where they are found forming a thick layer as shown in figures 5 and 6a. The shifted vesicles are still much larger in size than the globules.

At the stage when the germinal vesicle nears its migration, the globules attain conspicuously large size and are now no less in dimension than the vesicles. Therefore, in Ciaccio preparations it becomes difficult to demonstrate under low magnification the presence of the vesicles situated in the peripheral ooplasm (Fig. 7). During the prematuration and maturation stages, the fusion of the yolk globules seems to occur. The globules grow much in size but decrease in number. Now the globules are much larger in size than the vesicles. It is noteworthy that the affinity of the globule for Sudan III or Sudan IV decreases clearly and the fatty droplets, which were stained with Sudan III and dissolved out with fat solvents, make appearance in the ooplasm during this period. In Ciaccio preparations the globules and follicle layer gave a positive reaction, while the cytoplasm, yolk vesicles and egg membrane were always negative (Fig. 8). Even in ripe eggs, there are found many yolk globules of large size, but no yolk in a continuous mass.

2. The chemical nature of lipids present in the yolk globules

As already mentioned above, the yolk globules of herring oocytes were strongly Ciaccio-reactive throughout vitellogenesis. To investigate the chemical nature of lipids present in the globules, the oocytes in the secondary yolk stage were subjected to several cytochemical tests following Lison's table for lipid analysis (1953). The results obtained are as follows: The modified Sudan black B and alcohol method clearly demon-

strated the presence of lipids in the globules, being stained deeply in blue black (Fig. 6B). Unstained formalin-fixed frozen sections were almost colourless and proved to contain no discernible carotinoids. Schultze's test for cholesterol was also negative. By the application of Baker's test for phospholipids, the globules were deeply stained blue black. In contrast to the globules of the flounder, however, the yolk globules of the herring oocytes were stained blue black even after being treated with pyridine, although some reduction of coloration could be detected under the treatment (Figs. 9a and 9b). The globules were stained blue by Smith's Nile blue staining for the differentiation of blue-stained acid lipoid from red-stained neutral fat (Fig. 10).

From the data above, it is evident that the globules of the herring oocytes contain no appreciable quantities of glycerides and carotinoids and cholesterol as well as those of the flounder, but noteworthy it is that the yolk globules of the herring oocytes showed a positive reaction for both the standard acid haematein test and pyridine extract test. Cain (1947) reported a similar fact that the epidermal cells and plasmosomes of *Glassiphonia* give a positive reaction for both the standard acid haematein test and pyridine extraction test. From the detailed examination with Baker's test, he concluded that a substance which reacts positive for both the acid haematein test and pyridine test cannot be regarded as a phospholipid, and that only a blue-black coloration given by the acid haematein test but not by the pyridine extraction test indicates phospholipids. Therefore, the results obtained in the present study are apt to lead to the conclusion that the main part of the lipids demonstrated in the globules of the herring must be some kinds of conjugated lipids other than phospholipids. However, it seems necessary to undertake further detailed examinations before this conclusion may be fully accepted, because the specificity of the Baker's test depends on the relatively greater affinity of phospholipids among conjugated lipids for the mordant (Cain, 1947), whereas the globules are certainly composed of complicated lipid-protein complexes. Consequently, the resistance of the lipids in the globule against the fat solvents depends not only on the affinity of lipids for the mordant, but also on the quantitative difference of the constitutional components and the combining state of lipid-protein components. In this regard, together with taking into consideration biochemical results showing that fish eggs contain a large quantity of phospholipids but a meagre amount of other conjugated lipids (Young and Phinney 1951, Igarashi *et al.* 1955, 1956a, b), it seems justifiable to surmise that a weak reduction of coloration of pyridine extracted preparations does not necessary indicate the presence of a small amount of phospholipids, or rather the resistance of the lipids against pyridine extraction.

DISCUSSION

Concerning the formation of non-massed yolk of fish eggs, Lams reported detailed observations on *Osmerus eperlanus* as early as 1903. In *Osmerus*, fatty globules

appear as primary vitelline element. After the fatty globules have been formed into two layers, inner and outer, the yolk globules begin to be accumulated in the cytoplasm between the egg membrane and the fatty globules. The formation of the yolk globules then proceeds towards the interstices between the fatty globules of the outer layer, and finally all ooplasm between the fatty globules comes to be filled with the yolk globules. Lams' findings on *Osmerus* agree with the writer's findings on *Clupea* in so far as the formation of yolk globules is concerned, that is, the globules appear at first in the periphery of the ooplasm and are formed centripetally. But there can be found a marked difference between the two species. In *Osmerus*, fatty globules composed of two layers have already been accumulated in the inner and outer regions of the ooplasm before the yolk globules begin to be formed, while in *Clupea* only yolk vesicles have been found prior to the formation of yolk globules and the appearance of fatty droplets is recognized in far more advanced stages. However, as Lams did not make sure of the fatty nature of the globules by reliable techniques, it seems necessary to confirm whether the fatty globules are really fatty in nature or whether they correspond to the yolk vesicles composed mainly of mucopolysaccharides. Furthermore, the findings on the closely related species such as *Hypomesus japonicus* (K. Yamamoto 1955b) show that only yolk vesicles have been found in the outer regions of the cytoplasm before the formation of yolk globules begins to start.

Another striking report concerning the formation of non-massed yolk is that of Konopacka (1935) on *Cyprinus* and *Gobio*. Her findings fit in pretty well with those of the present writer. In these species as well as the present material, yolk vesicles which were designated by her as "gouttes claires" are firstly formed in the ooplasm of a peripheral region and then accumulated inwardly. Soon after, the granules of small size make appearance in the peripheral cytoplasm and then in the interstices between the vesicles. Along with the proceeding of vitellogenesis, these granules increase in size and number, and grow into yolk globules designated as "plaquete vitelline". During the later phase of vitellogenesis, one noteworthy difference between her materials and the herring can be recognizable. In *Cyprinus* and *Gobio* the vesicles situated in the inner part of the ooplasm, exclusive of the peripheral region, break down and the material contained in the vesicles play a supplementary part in the formation of the yolk globules; in *Clupea* the vesicles of the inner ooplasm do not break down and are only shifted towards the periphery of the oocyte as mentioned above. It is difficult to determine with certainty whether all vesicles present in the inner part of the ooplasm are shifted intact towards the periphery of the oocyte or not, because of the difficulty in preparing good sections from the advanced stages of fish eggs. But it seems improbable that most vesicles situated in the inner part break down and participate in the formation of the yolk globules. If that were true, the cortical layer could not be

embedded thoroughly with cortical alveoli as is really seen in ripe eggs, because the surface of the oocyte increases enormously with the growth of the oocytes, while the formation of the vesicles comes to an end in earlier stage and the number of the vesicles remains unaltered thereafter as confirmed by Konopacka (1935), K. Yamamoto (1956), and Osanai (1956). On the basis of the above considerations, therefore, it is most reasonable to consider that the yolk vesicles scarcely participate in the formation of the non-massed yolk, but give rise to the cortical alveoli by shifting towards the periphery of the ooplasm as already asserted by the present writer (1956) and Osanai (1956).

The presence of lipids in the non-massed yolk of fish eggs has already been demonstrated by Konopacka (1935) working on *Gobio* and *Cyprinus*, by Mas (1952) on *Perca*. On the other hand, the presence of lipids in the globules has also been established in *Oryzias* (T. S. Yamamoto 1955) and *Liopsetta* (K. Yamamoto 1957) whose globules give rise to a continuous mass of yolk. Therefore, the findings obtained in the present study offer further evidence for the conclusion that the yolk globules of fishes, regardless of whether or not they give rise to a continuous mass of yolk, contain a large quantity of lipids. However, the ratio between lipids and proteins in yolk globules is not always similar in all species and in all yolks at different stages. In *Gobio* and *Cyprinus* the yolk globules at the beginning of formation are composed mainly of lipids and the lipid components of the globules then come to combine with protein substances as yolk formation proceeds (Konopacka 1935); *Liopsetta* globules, judging from the result of Sudan staining tests, become rich in lipids with the growth of oocytes (K. Yamamoto 1957). On the other hand, the globules of the herring oocytes contain much lipids from the beginning of formation and the lipid-protein ratio in the globules appear to remain almost unchanged until the time when fat droplets are formed.

As to the quality of lipids in the globules, Konopacka (1935), from the result of Smith-Dietrich test, asserted that the lipids in the yolk globules of *Gobio* and *Cyprinus* are phospholipids. A similar conclusion was obtained by the present writer, using the Baker's test and Smith's Nile blue staining test, that the globule lipids in the flounder must be phospholipids composed mainly of lecithin, but the substance seems to be changed in nature during vitellogenesis. On the other hand, the yolk globules of herring oocytes showed a different reaction in response to the Baker's test giving only a weak reduction of coloration for the pyridine extraction test. But this difference in reaction is considered to depend partly upon the difference of the nature of lipid itself, but rather more upon the quantitative difference of constitutional components and the combining state of lipid-protein components in the globules. Taking into consideration the results of biochemical analysis, therefore, it seems probable that the globule lipids of fish eggs consist mainly of phospholipids.

SUMMARY

1. The formation of yolk globules in the herring oocytes commences in the periphery of the ooplasm and proceeds inwardly until a greater part of the ooplasm is filled with them. At the last stage of vitellogenesis, the globules become large in size by their fusion, but make no continuous mass of yolk. The yolk vesicles appear to play no role in the formation of the yolk globules.

2. The presence of lipid is established in the yolk globules of all stages by cytochemical techniques. The globules in the oocytes of the secondary yolk stage showed a weak reduction in response to the Baker's pyridine extraction test, but they are supposed to be composed mainly of phospholipids.

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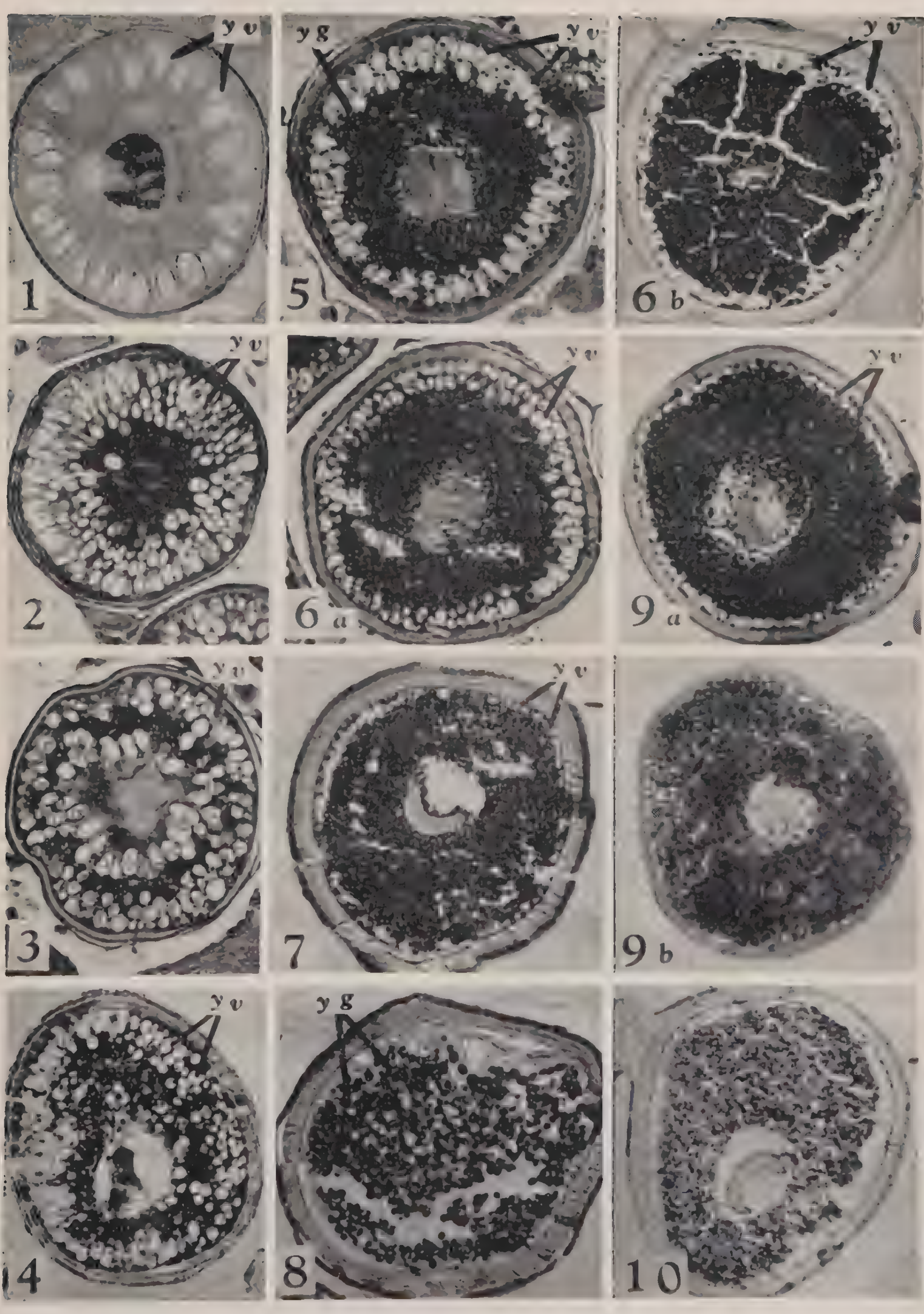
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Explantion of Plate

All figures are photomicrographs from sections of herring eggs.

Figs. 1. and 2. Yolk vesicle stage. Regaud and Sudan IV preparations.
Figs. 3. and 4. Primary yolk stage. Preparations as above.
Fig. 5. Secondary yolk stage. Regaud and Sudan III preparation.
Fig. 6a. Same stage as above. Regaud and Sudan IV preparation.
Fig. 6b. Same stage as above. Modified Sudan black B and alcohol preparation.
Fig. 7. Tertiary yolk stage. Regaud and Sudan IV preparation.
Fig. 8. Maturation stage. Preparation as above.
Fig. 9a. Secondary yolk stage. Baker's acid heamatein preparation.
Fig. 9b. Same stage as above. Baker preparation with pyridine treatment.
Fig. 10. Same stage as above. Smith's Nile blue staining preparation.
y.v. yolk vesicle, y.g. yolk globule.



K. YAMAMOTO: Formation of Fish Eggs (XII)

紅藻ツルツルの果胞子発芽体の発達について

籔　　　　凞

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On the Development of Carpospore-germlings of *Grateloupia turuturu* YAMADA

Hiroshi YABU

Abstract

As regard to the embryonal development of *Grateloupia turuturu* YAMADA, a result of cultural experiments of the carpospores was once reported by Inoh (1947, p. 133-135, Fig. 115), whose description was limited to a short period of early development up to the time of formation of small disk-shaped embryos six days old. The present research was carried out from August of 1954 to December of 1955 in our laboratory with the object of knowing further developed embryonal stages of this alga under various conditions of the substratum. The materials employed as a substratum were ordinary microscopic slide-glass, frosted glass, shell pebble, and chopstick. The carpospores germinated on one of these materials develped in a somewhat different way as compered with those on others. In a culture more than six months old, monosiphonous colourless filaments were often observed to have grown out from the surface of the disk-shaped embryos. On these filaments were produced disk- or mulberry-shaped bodies, of which the former gave rise on their surface to one to several upheavals or initials of the erect fronds just as the disks derived directly from the spores did after 8 to 9 months since the carpospore germination. Monosiphonous filamentous outgrowths here described are believed to have been unknown to *Grateloupia* if the hyaline hairs illustrated briefly by Inoh (1947, p. 136, Fig. 117) in *Grateloupia elliptica* HOLM. are not identical with them. These filaments were also produced from the upheaved center of a disk-shaped embryo derived from a mulberry-shaped body wich had been cultured separately and had become attached to a slide-glass. These filaments sometimes gave rise to a few fine monosiphonous filaments which also produced disk- or mulberry-shaped bodies on them when cultured separately. In view of the result of this research, multiplication of this alga in nature is supposed to be favoured by repeated formation of the secondary disk- or mulberry-shaped embryos.

1. 緒　　　言

ツルツル（*Grateloupia turuturu* YAMADA）は東北，北海道沿岸で夏期普通にみられる紅藻である。本種の発生に関しては猪野（1947）による果胞子培養の研究があるが，氏の研究は果胞子が盤状の発生体となるまでの極く初期の発生過程を観察したものであり，盤状体からの直立体形成はタンバノリでは見ているが本種では記述がない。筆者は1954年８月から数種の異つた基質上に本種の果胞子を培養したところ，附着する基質によつて発生体の形態がかなり異つてくるのを認めたほか，果胞子から発生した盤状体上に直立体が形成されるのを観察した。更に長期間培養した盤状体からは無色の単列細胞の枝を生じてきたが，この枝の上にも盤状又は桑実状の体が形成された。又，この単列細胞の枝，並びにその上に形成された桑実状体を培養した結果，二三の興味ある事実が観察されたので，茲に報告する次第である。本文に入るに先だち本稿の御校閲を賜つた恩師時田郇教授に深謝の意を表する。

2. 種々の異つた基質上での果胞子の発生

材料と方法

材料は1954年8月18日に函館七重浜の第三防砂堤で採集したもので，濾過海水でよく洗つた後，深さ15cm径7cmのガラス容器の中で胞子を放出させた。容器の中に予め普通のスライドグラス，スリガラス，貝殻，海岸で拾つた小石，充分水を吸収させて培養液中に浮かばないようにした割箸等を置き，これに胞子を附着させた。培養液は濾過海水を単用し，容器一杯に満たし，培養中そのまま取り換えず，ただ蒸発により容器の4/5位まで減つた時に新しい濾過海水をもとの量になるまで注加した。培養は実験室内で行い液の温度調節等は行わなかつた。

観察結果

胞子の発生初期は，小石や割箸上のものは検鏡が困難で詳細は明らかでないが，スライドグラス，スリガラス，貝殻上のものの観察は猪野の報告と一致し，培養10日頃には発生体は20〜30個細胞からなる盤状体となる。しかし，異状発生体もみられ，基本細胞が細長くなつて糸状の発生体となつたもの，数個の盤状発生体が癒合したもの(Fig. 1,C)及び分岐した糸状体も見られた。培養20日の糸状を呈する異常発生体は殆んど先端に盤状体を形成し始め (Fig. 1, A, B) その後発生が進むに従い正常な発生体と同じく盤状体の周辺の細胞は色のうすい帯色体を有するものとなる。培養開始後3ケ月を経過した11月中旬には，基質によつて発生体の形態に相違が認められてきた。即ち，スライド上で発生体が密生しているところでは体が互に癒合しているものもあるが，まばらに附着している場所の発生体はそれぞれ完全な円形を呈している。ところが貝殻上では不規則な円形を呈し，割箸上では木質纖維組織内で成長し5〜6個細胞の列から成る細長い体となつていた。一方，小石の上では，平滑な面ではほぼ完全な円形を呈するが，凹部や割れ目ではその内部だけに存して不規則な形を呈するものが多い。培養6ケ月を経た翌年2月に至ると，基質による発生体の形態の相違は益々顕著となつてきた。同じ貝殻上でも表面と裏面では，形態に著しい相違が認められた。即ち，裏面に附着する体は様々な形の叉状に分岐しており，その多くの細胞からは細長い無色の単列細胞から成る枝が一本ずつ出ている (PL. I, 1)。表面に附着する体は著しく叉状に分岐したものは見られず，円形がやゝくずれた程度のものが多いが，単列細胞の枝は同じ様に観

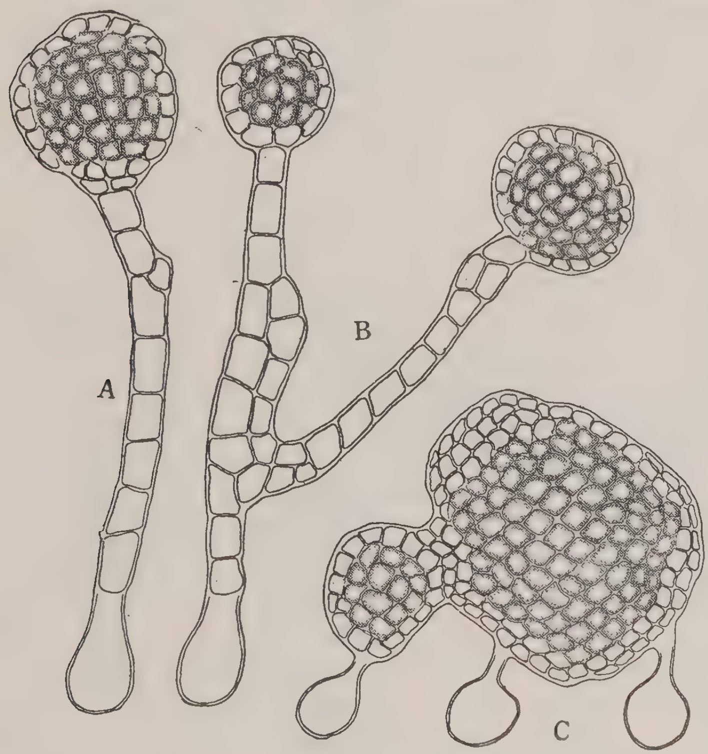


Fig. 1. Disk-shaped embryos of *Grateloupia turuturu* Yamada developed from carpospores directly or indirectly. ×550. A, a disk formed at the tip of a filamentous germling from a culture 21 days old; B, a disk formed at each tip of a branched filamentous germling from a culture 21 days old; C, a compound disk which formed by the coalescence of three contiguous disks each developed directly from a carpospore.

察された。此の枝は分枝し，顕著な屈光性を示す。PL. I, 2は此の時期の貝殻の極く薄い部分の表面に附着する体とその上に生ずる単列細胞の枝とを示す写真で幾分薄く写つている奇妙な形態のものは裏面に附着している体である。貝殻の縁に附着している体は，その端まで生長が進むと，そこからは無色の単列細胞の枝を形成し，それは基質から離れて伸びていく（PL.I,3）。この時期に於ける割箸上の体は纖維組織内で生長をつづけているが，纖維組織の側壁が破れている処では，そこから体が外に進出し，隣の纖維細胞内でまた生長をつづける。これを繰返した後，やがて隣接する体の間に接着癒合が起る。PL. I, 6は此の状態を示し，割箸を薄く剝いで撮影したもので白く見えるのが割箸の切片であり，その中にうす黒く粒状物が集つて見えるのが発生体である。単列細胞枝の形成は此の体の上にも見られたがその数は非常に少なかつた。この時のスライドグラス上の発生体は，まばらに附着のものは培養3ケ月のものと同じく完全な円形を呈するが，周辺から中心に至る½位の間を構成する細胞は内容物が乏しく，帯色体の数も少なく，色もうす桃色を呈していた（PL.I,5）。又，発生体が密生する処では隣接する体は完全に接着癒合し接着部は屢々幾分もり上つて隣接する体の境界線が明瞭に観察された。PL. I, 8は癒合した体を示し，中央の斜め線状に見える箇所が接着部である。この発生体にも図にもみられる如く，単列細胞枝の形成が認められた。又，PL. I, 9は隣接する発生体が互に接着し始めたところと，発生体がスライド上の空所に生長して行く状態，並びにそれから単列細胞枝を伸長する様子を示したものである。更に1ケ月を経過した3月の観察では，これら単細胞枝上に小さな盤状体や桑実状体が形成されるのが認められた。単列細胞枝は母体から屢々切り離されて液中に浮游しているものや，培養器の側壁に附着生長しているものなどが観察されたが，何れの場合も盤状体，桑実状体を生じていた。盤状体は基質に附着して伸長する単列細胞枝上に，桑実状体は基質に附着せず培養液中に浮んで伸長している枝の上に見られた。これらの体の形成に先だち単列細胞枝中の細胞に帯色体が現われて細胞は紅色を呈するようになり，やがて分裂する。その分裂は不規則で，細胞列の方向に垂直，平行，又は斜めの方向に細胞膜が形成される。又，帯色体を形成した細胞からは稀に細い単列細胞枝を出しているのが見られた（Fig. 2, G, H；PL. II, 1, 2）この分裂する細胞が他の分裂する細胞と接する際には，両者は接着癒合して一体となり，1個の盤状体又は桑実状体を形成する。これらの体はその後生長を続けやがて体の周辺に帯色体の色のうすい細胞が形成される。培養開始後13ケ月を経た9月中旬には大きなものは径約 0.5mm となつたが，体の周辺部は全く無色の細胞から成つている。培養開始後8〜9ケ月を経過した4月中旬から5月中旬にかけて，果胞子に直接由来する盤状体の上に直立体の形成が見られ，何れの基質上のものにも同じように形成された。まばらに附着した体では直立体は中央に1個形成されるのが普通であるが，比較的大きな盤状体では稀に2乃至数個の直立体が見られた。その場合，盤状体中央部の帯色体の色の濃い部分に限つて形成され，色のうすい部分や周辺部の無色の部分には直立体の形成はみられない。PL. II, 5は貝殻上の盤状体の表面観で，黒色円形の部が直立体である。PL. II, 6は同上の直立体の側面観を示す。7月中旬に至ると直立体は順次枯死し始め，盤状体から離脱し，其のあとに円い空所が残される（PL. II, 7)。この空所はやがてその周囲から生ずる無色の細胞で満たされるが，9月中旬の観察ではその多くは帯色体を有するようになつており，やがて周囲の細胞との識別がつかなくなる。

3. 単列細胞枝，並びに單列細胞枝から形成された桑実状体及び細い細胞枝の分離培養

上に述べた如く，果胞子からの発生体は無色の単列細胞枝を形成し，その上には盤状又は桑実状の体を形成するほか，細い枝をも生じたが，これらを分離して培養してみた結果は次の通りである。

a. 單列細胞枝の培養

分離培養に供した単列細胞枝は果胞子から発芽後6ケ月を経た発生体の貝殻の縁辺に附着するものから形成されて基質から離れて液中に伸長していたものである。

分離に際しては桑実状体のまだ形成されないもの及びその形成の前ぶれでるあ帯色体を有する細胞のまだ認められない枝だけを取り出し，数十個細胞から成る部分に切断して Schreiber 氏液で培養した。

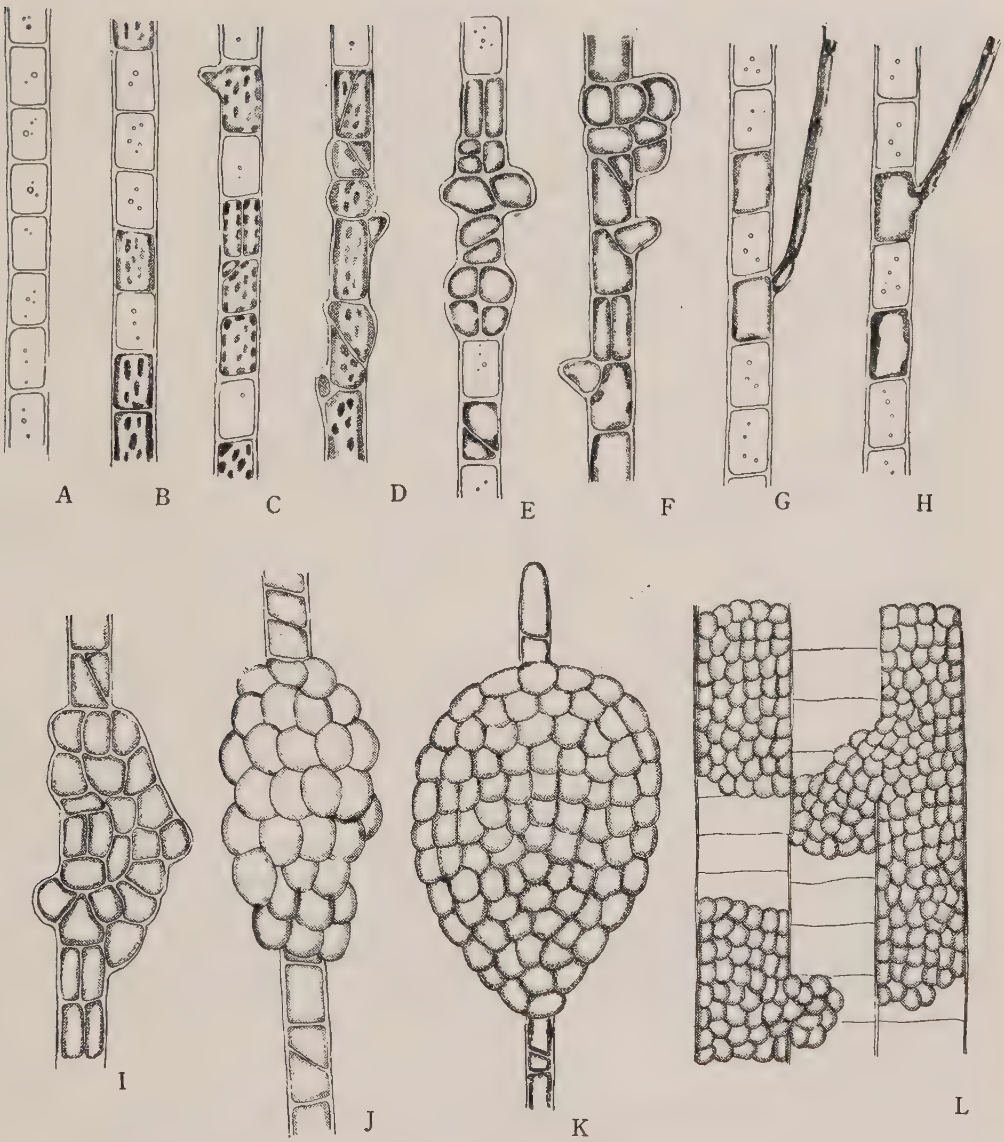


Fig. 2. Camera lucida drawing of carpospore-germlings of *Grateloupia turuturu* YAMADA. A, part of a colourless monosiphonous filament borne on a diskshaped embryo developed from a carpospore; B & C, part of a monosiphonous filament showing that some of the cells contain chromatophores; D–F, part of a filament showing the begining of divisions in some of the intercalary cells; G & H, part of a monosiphonous filament bearing a slender monosiphonous outgrowth; I, a disk-shaped body at an early stage of its formation on a monosiphonous filament; J & K, a mulberry-shaped body at an early (Fig. J) and an advanced stage (Fig. K) of its development on a monosiphonous filaments; L, three carpospore-germlings developed within the vascular cells of a chopstick, two of which protrude partly into a adjacent vascular cell through a tear on the wall. From a culture 6 months old.

Magnification: A–J, × 840; K, × 610; L, × 480.

培養液中に入れたものは，はじめ浮んだまゝで無色の細胞が増加して伸長を続けるが，やがて体の一部が基底に附着するようになる。分離後1ケ月経つと多くの細胞に帯色体の形成がみられ，細胞は分裂して桑実状体又は盤状体が形成される。その過程は果胞子から発生した体の単列細胞枝のものと一致する。分離後4ケ月経た6月中旬には少数の盤状体の上に小さな直立体の形成がみえ始めた。盤状体の中央に1個形成されるのが普通で，稀に2個形成される。この直立体は7月中旬には枯死し，基部から離脱し，そのあとに空所が残つているのが見られ，のちに其の周囲の細胞から生じた無色の細胞で埋められる。

b. 單列細胞枝に形成された桑実状体の分離培養

桑実状体を分離するには単列細胞枝の先端又はその附近にあるものをえらび，分離してからついている単列細胞枝の残りをピンセットで出来るだけ取り除いた。合計7個を分離し，濾過海水で培養した。

分離後2週間を経過した体のうち2個はすでに器底に附着していたが，他の固体はまだ附着せず，器を動かすと液と共に動く状態にあつた。

分離後半月から1ケ月位の間に殆んどの体は無色の単列細胞枝を数本ずつ生じ，これらは伸長分枝し，一部は器底に附着して伸長する。液中に遊離して生長する枝には桑実体，器底で伸長する枝には盤状体が形成された。

分離直後器底に附着した2個の桑実体の内，1個は分離後2ケ月を経過しても単列細胞枝の形成がみられず，細胞の分裂をつづけ器底に附着したまま盤状体となり，やがて小さい山型の体となつた。これは分離後5ケ月には径0.5mmの体となり，中央凸部の桑実状を呈する部分に少数の無色の単列細胞枝が形成されているのを認めた。

c. 單列細胞枝から分枝した細い細胞列の分離培養

貝殻上で発芽した果胞子の発生体に形成された単列細胞枝から分枝した細い細胞列を分離培養してみた。分離は果胞子培養開始後8ケ月を経過した4月中旬に行つた。培養は濾過海水で行い，容器中には十数本ずつの細胞列を入れた。この細胞列は十数個又は数十個の細胞から成り，その細胞は径約4〜5μ，長さは径の約3〜4倍あり，細胞列の先端又は途中に稀れに無色の細胞を有するものがあつた。培養液（濾過海水）中でよく伸長分枝し，分離後3ケ月には稀に太い細胞が見られ，これは分裂して盤状体を形成した。此の盤状体は細胞数を増し，大きくなつたが，体の中心部の細胞，即ち形成初期の数個乃至十数個細胞にのみ帯色体がみられ，その他の細胞は無色であるのが普通であつた。分離後6ケ月を経た観察では，盤状体の細胞のうち帯色体を有する細胞が十数個乃至数個ずつかたまつて散在する状態のものがみられた。盤状体は光に向つて生長する傾向がある。盤状体の比較的大きなものは器底から容易に剝がすことが出来た。PL. Ⅲ，5 の白色膜状の物は器底の他の場所から剝いだものである。培養は分離後8ケ月経た12月中旬迄行つたが盤状体上に直立体の形成は見られなかつた。

4. 論　　議

ツルツルの属するムカデノリ科の発生については猪野（1947）による本種の果胞子およびムカデノリ（*Grateloupia filicina* (Walf.) Ag.）の果胞子と四分胞子の培養，並びにタンバノリ（*Grateloupia elliptica* Holm.）の果胞子の培養結果の報告があり，直立体の形成についてはタンバノリ（P. 136，図117）に簡単な記載がある。発生体の上に単列細胞枝を形成することも記述されていない。タンバノリでは猪野は胞子放出後20日位を経過した発生体の表面から無色の，数本の毛状突起を生ずるのをみているが，筆者はツルツルでかかる突起を見なかつた。筆者の培養では果胞子の発生体は基質の相違によつてかなり異つた形態を呈することを観察した。海中では基質は一様でないから，発生体はおそらく様々な形態をとり，筆者の培養で観察したと同じように発生体に単列細胞枝の形成が行われ，これにも盤状体を生じ，その上にも直立体が出来て個体の増殖が盛んに行われるものと想像される。

5. 摘　　要

1. 紅藻ツルツルの果胞子を種々の異つた基質上に附着させその発生について観察し，更に果胞子の発生体上に生ずる無色の単列細胞枝並びにそれを分離培養した結果について記し，これらの観察に基き自然の海中に於ける発生状況を考察してみた。
2. 培養基質としてはスライドグラス，スリガラス，貝殻，小石及び割箸を用いた。発生体は基質によつてかなり異つた形態を呈した。
3. 果胞子から直接発生した盤状体の上に直立体の形成が認められた。直立体は盤状体の中央に1個形成されるのが普通であるが稀に2個乃至数個形成されたものも見られた。
4. 直立体は培養では或る程度の大きさになると枯死し，盤状体から離脱する。そのあとには空所が残るがこの空所はのちにその周囲から生ずる細胞で埋められる。
5. 果胞子から発生した体は長期間培養すると無色の単列細胞枝を生じ，その上に盤状又は桑実状の体が形成された。
6. 単列細胞枝上に盤状又は桑実状の体が形成される時には先づ単列細胞枝中の或る細胞に帯色体が現われた後分裂が行われる。その分裂の方向は一定しておらず，分裂体が互に接すると体は癒合して一体となる。
7. 単列細胞枝に生じた盤状体上にも直立体の形成が見られた。

文　　献

1) 猪野俊平 (1947). 海藻の発生. 255 p. 東京；北隆館.

PLATE I

Microphotographs of carpospore-germlings of *Grateloupia turuturu* YAMADA

Fig. 1. Germlings attached to the surface of a shell in a 6 months old culture. × 40.

Fig. 2. Germlings on the upper surface of a shell in a 6 months old culture. Germlings on the other side of the shell are seen in the photograph as faint shadows. × 80.

Fig. 3. Monosiphonous filaments growing into the medium from the disk-shaped germlings on the edge of a shell × 80.

Fig. 4. Floating mass of monosiphonous filaments detached from the disk-shaped germlings on the edge of a shell. × 80.

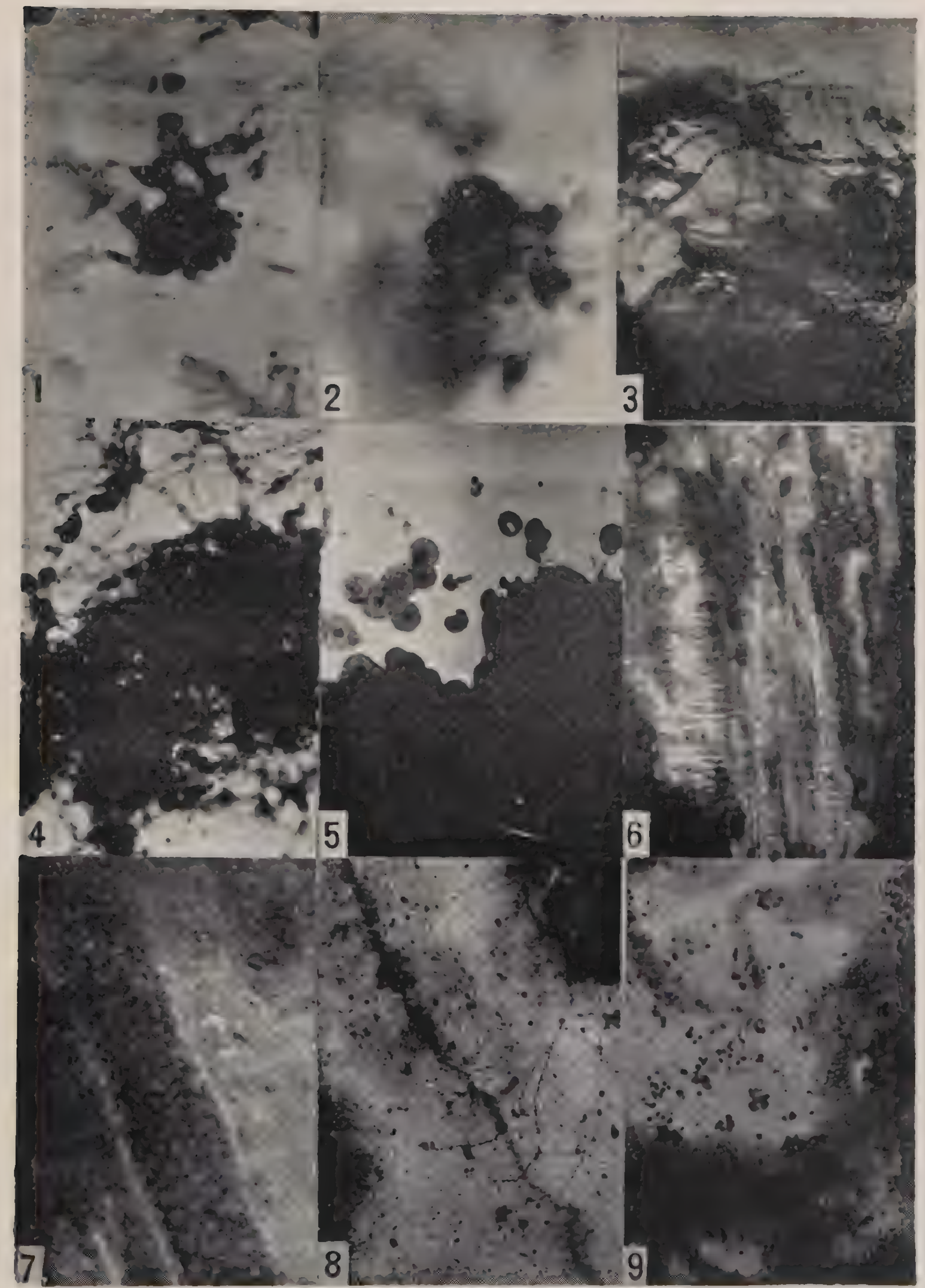
Fig. 5. Gelmlings on a slide-glass in a 6 months old culture. × 3.

Fig. 6. Germlings on a piece of chopstick in a 6 months old culture. × 80.

Fig. 7. The same preparation as shown in Fig. 6, enlarged. × 320.

Fig. 8. Part of the seamed portion of two contiguous disk-shaped germlings on a slide-glass in a 6 months old culture; a number of monosiphonous filaments on the germlings are seen in the photograph. × 80.

Fig. 9. A space among a few contiguous disk-shaped embryos on a slide-glass. × 80.



H. YABU: On the Development of Carpospore-germlings of *Grateloupia turuturu* YAMADA

PLATE II

Microphotographs of carpospre-germlings of *Grateloupia turuturu* YAMADA

Figs. 1 & 2. Monosiphonous filaments produced from the germlings on the edge of a shell in a 8 months old culture; mulberry-shaped bodies and slender filamentous outgrowths are found on these filaments. × 320.

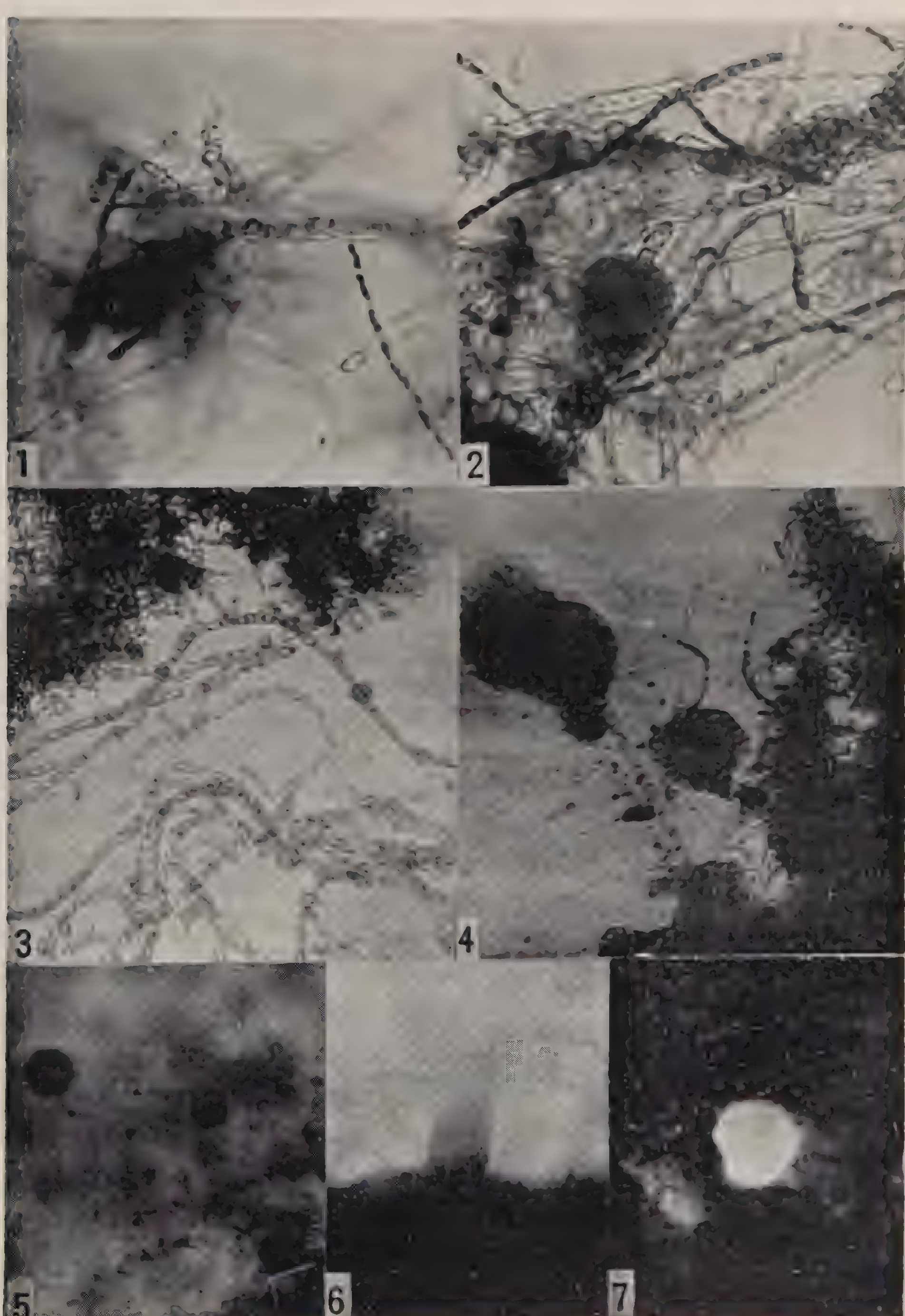
Fig. 3. Monosiphonous filaments and well developed slender filamentous outgrowths on a slide-glass in a 10 months old culture. × 150.

Fig. 4. Disk-shaped bodies formed at the tips of monosiphonous filaments, in a 10 months old culture. × 150.

Fig. 5. Surface view of disk-shaped germlings on the upper surface of a shell in a 10 months old culture, showing several upheavals or intitials of the erect fronds which are seen in the picture as dark disks. × 80.

Fig. 6. Side view of an upheaval in a culture one month older than that shown in Fig. 5. × 80.

Fig. 7. Part of a surface view of a disk-shaped embryo showing a roundish empty space left behind after an upheaval had died and detached away. × 80.



H. YABU: On the Development of Carpospore-germlings of *Grateloupia turuturu* YAMADA

PLATE III

Microphotographs of carpospore-germlings of *Grateloupia turuturu* YAMADA

Fig. 1. Monosiphonous filaments bearing disk- and mulberry-shaped bodies 2 months after they had been separated and cultured with Schreiber's solution. × 80.

Fig. 2. Pieces of monosiphonous filaments bearing mulberry-shaped bodies which had been cut for the purpose of culturing those bodies in separation. × 80.

Fig. 3. A mulberry-shaped body one week after it had been separated from a monosiphonous filament; four short monosiphonous filaments are seen growing out from the body. × 320.

Fig. 4. Mass of well developed slender filaments 8 months after they had been separated. × 18.

Fig. 5. Mass of slender filaments 7 months after they had been separated, and parts of colourless disk-shaped bodies produced from those filaments. × 80.

Fig. 6. Mass of well developed slender filaments 6 months after they had been separated from the monosiphonous filaments on disk-shaped embryos. × 120.

Fig. 7. Monosiphonous filaments developed from a mulberry-shaped body which had been separated 2 months before. The filaments are growng toward the light; they are repeatedly branched, and bearing mulberry-shaped bodies. × 80.

Fig. 8. Well developed slender filaments and a disk-shaped body produced from them, 6 months after they had been separated. × 320.

Fig. 9. The same photograph as Fig. 8; the arrow points to a group of chromatophore-bearing cells which was made by shading to contrast with other colourless cells of the disk-shaped body.



H. YABU: On the Development of Carpospore-germlings of *Grateloupia turuturu* YAMADA

エゾヤハズとアミヂグサの四分胞子嚢に於ける核分裂について

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On the Nuclear Division in Tetrasporangia of *Dictyopteris divaricata* (OKAMURA) OKAMURA and *Dictyota dichotoma* LAMOUR

Hiroshi YABU

Abstract

This paper deals with the nuclear divisions in the tetrasporangia of *Dictyopteris divaricata* (OKAMURA) OKAMURA and *Dictyota dichotoma* LAMOUR collcted in Oshoro Bay, Hokkaido. In the former were observed normal reduction divisions in the first nuclear division, and 16 vivalent chromosomes were counted in diakinesis and 16 chromosomes in anaphase of the first and second nuclear divisions. In *Dictyota dichotoma*, 32 chromosomns were counted in early metaphase of the first and second nuclear divisions, and also in metaphase of somatic divisions in the cells of the plantets which were found growing on the surface of tetrasporic plants having no doubt developed abnormally from tetrasporangia or tetraspores.

The two species here investigated, as compared with each other, show several noticable differences in the first nuclear divisions of the tetrasporangia as follows: The so-called "spherule" was very often observed in the nuclear cavity of *Dictyopteris divaricata* in early prophase, but not in *Dictyota dichotoma*; chromatin threads formed at one side of the nuclear cavity were somewhat broder in size and more deeply stained in *Dictyopteris divaricata* than in *Dictyota dichotoma*; in *Dictyopteris divaricata*, the nuclear membrane was still present in the metaphase, centrosomes were not found, and the spindle was stained well, while in *Dictyota dichotoma*, the nuclear membrane was already absent in the metaphase, cenrosomes were sometimes found, and the spindle was scarcely noticed because of its faint stain.

エゾヤハズ (*Dictyopteris divaricata* (OKAMURA) OKAMURA)の細胞学的研究としては猪野（1936）による報告があり，四分胞子が発芽して生ずる仮根細胞の核分裂像でn＝16の染色体数を算えている。しかし，四分胞子形成については母細胞内の核が4個の小核に分裂して胞子が形成されると簡単に記述するのみで，核分裂の詳細は未発表となつている。筆者は本種の四分胞子嚢に於ける核分裂の過程を観察することが出来た。又，アミヂグサ (*Dictyota dichotoma* LAMOUR) については既に WILLIAMS (1909) の研究があるが，邦産の材料についての報告はまだない。筆者はエゾヤハズの核分裂と比較のためアミヂグサもしらべてみたが，両種の四分胞子嚢内の核分裂には注目すべき差異が認められたので，茲にこれらの結果を報告する次第である。

本文に入るに先立ち本研究に当つて材料の固定その他について御指導戴いた本学部動物発生学教室，新山英二郎助教授並びに本稿の校閲を辱くした時田郇教授に深謝の意を表する。

1. 材料と方法

材料としたエゾヤハズ，アミヂグサはいづれも1956年と1957年の7月中旬，北海道忍路湾で採集し，同地の北大臨海実験所研究室で海水を入れた容器の中に生かしておき適当な時間に固定した。固定は阿部氏液（阿部，1936）と海水を用いたナワシン氏（液1％クロム酸海水液10cc，氷酢酸1cc，ホルマリン4cc）とで行い，固定時間は6～12時間とした。パラヒン法により切片の厚さは8～12μとし，染色はハイデンハイン氏

鉄ヘマトキシリンで行つた。細胞質並びに核は上記の両固定液でよく固定されるが阿部氏液の方が鮮明な核分裂像が得られた。

2. エゾヤハズの四分胞子嚢に於ける核分裂

A．四分胞子母細胞とその柄細胞の形成

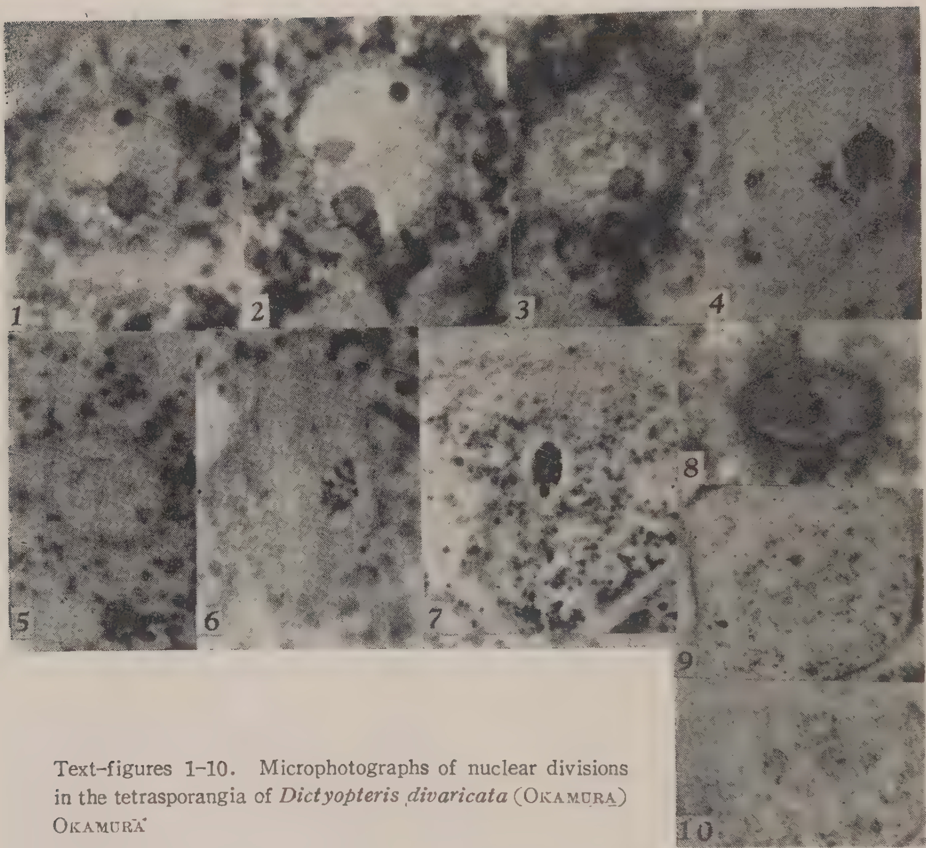
体の表皮細胞内にはうすく青色に染まつた1個の小さな核が見え，その中に1個の仁がある。四分胞子形成に際しては先づ一つの表皮細胞が大さくなり，体表面に平行な膜で2分され，四分胞子嚢とその柄細胞とになる。この場合，大きくなり始めたばかりの表皮細胞の核は既にその色は消え，核内には多数の小染色粒がみられ，次いで染色糸が形成される。この細胞の径が表皮細胞の厚さの2〜3倍となつた時，核分裂が行われる。分裂中期では仁は消失するが核膜は認められる。中期側面観では染色体は核板に並び，紡錘糸が認められるが中心体らしいものは見当らない。分裂は体表面に直角に行われる。後期で染色体群が両極に移動し，2個の娘核が形成されると，核膜と仁が再び現われる。其の後，2娘核のうち，細胞の基部に近い方の核は小さくなり，基部の方に移動し，体表面と同じ位の面，又はそれより低い面に隔膜が形成され，ここに細胞は四分子胞母細胞と柄細胞とに分たれる。往々，柄細胞は更に分裂して四分胞子母細胞が分裂を始める前に既に2〜3個の細胞となつているものが見られた。

B．四分胞子嚢の分裂

四分胞子母細胞は大きさを増し，核も大きくなりながら細胞の中央に移動する。同時に核腔内にはうすく染まつた細い核糸が認められるようになる。仁は次第に染まりが不均等となり網目状を呈し，屢々内部に空胞がみられる。間もなく核内には少数の染色粒が現われ，やがてよく染まつた染色糸となる。染色糸は始め核内の一方に片寄つたループを形成するが，間もなく仁と接続し，次第に核腔内一杯に拡がつてくる。核分裂前期の始には核内には屢々仁のほかに仁よりも遙かに小さく，濃く染まつた小体が現われる。染色糸は屢々この小体に接続しているものがみられた。又，同じく前期の始めには稀に核内に2個の仁が存在することがあるが，かような核では染色糸は核内の一側部に並んだ2群のループを形成し，それらは夫々1個の仁に接続していることがある。核腔内に拡がつた染色糸は其の後やゝ染まりがうすくなり，2本ずつが対をなすようになる。次いで染色糸は次第に短かく且つ太くなり，2本ずつ対をなすよじれ合つた染色体となる。この頃には仁は小さな突起を形成し，稀に突起の先端が1本の染色糸に連結し，その部分から仁が次第に褪色していくものがみられた。又，染色体には螺線構造がみられることがある。diakinesis 期では16個の二価染色体が観察された。分裂中期には仁は消失し紡錘糸が現われるが両極には中心体は認められない。核膜は中期にはまだ見られるが，後期には消失する。後期の始めには両極に分れようとする16個の染色体が算えられた。第1回分裂の多くは胞子嚢の縦軸の方向に行われるが軸と直角又は斜めの方向にも分裂する。染色体が両極に移動する時にはそれをつなぐ連絡糸が観察された。両極に移動し終えた染色体群は極で球状となるが，この時には連絡糸は太くよく染まつて見られる。やがて紡錘糸は消え，核膜を生じ，2娘核が形成される。続いて第2回目の分裂が始まるが，その分裂前期の始めには稀に2個の仁を有するものが見られた。第1回核分裂同様，第2回分裂に於ても核膜は中期では残存し後期に消失する。又，第2回核分裂後期の始めに於ても両極に移動する16個ずつの染色体を観察した。第2回目の核分裂が終ると胞子嚢内には隔膜が形成され四分胞子が出来上る。稀に第1回核分裂が終ると胞子嚢内に核膜が形成され，続いて第2回目の分裂が行われるものもみられた。又，胞子の放出されたあとの空の胞子嚢の中に新たに柄細胞から新らしい胞子嚢が形成されているものが観察された。

3. アミヂグサの四分胞子嚢に於ける核分裂

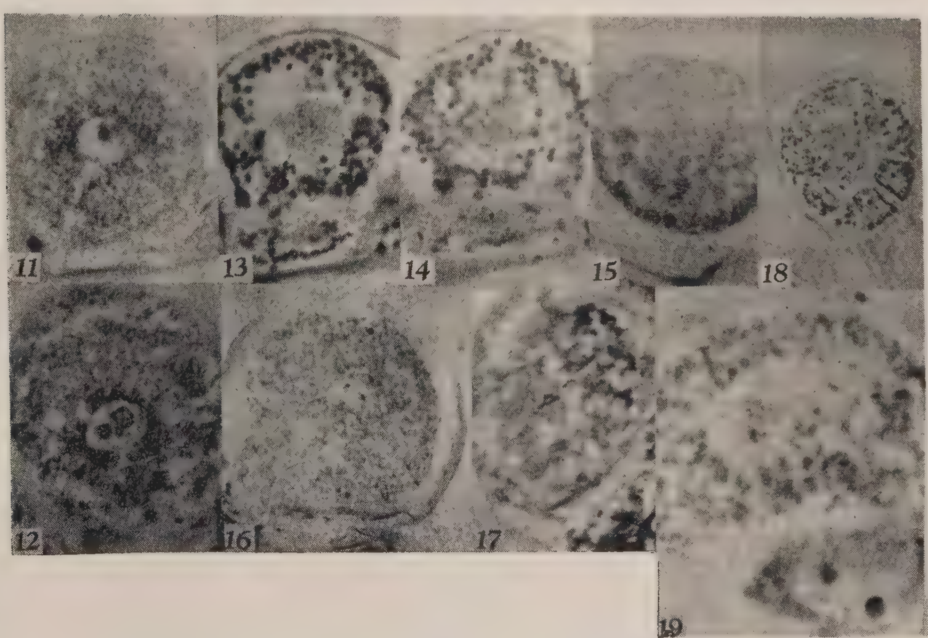
四分胞子体の表皮細胞から四分胞子嚢の形成される過程はエゾヤハズと略々同じであるが，核膜は核分裂中期に消失する。又，紡錘糸は観察されなかつた。若い四分胞子嚢内には1個の核がみられ，核内には1個



Text-figures 1–10. Microphotographs of nuclear divisions in the tetrasporangia of *Dictyopteris divaricata* (OKAMURA) OKAMURA

1. A nucleus in synapsis with grouped chromatin threads at one side of the nuclear cavity. (×1200) 2. A nucleus in synapsis, with two nucleoles, each of which is connected with chromatin threads. (×1200) 3. A nucleus in synapsis with chromatin threads spread in the whole nuclear cavity. (×1200) 4 & 5. Nuclei in diakinesis with vivalent chromosomes. (4.×1200; 5.×1000). 6. A nucleus in early anaphase; the same nucleus is shown in Pl. I, fig. 11. (×1400) 7. A distinct spindle between the poles in telophase of the first nuclear division; the same spindle is shown in Pl. I. Fig. 12 (×530) 8. Side view of a nucleus in the early anaphase of the first nuclear division; the same nucleus is shown in Pl. I. fig. 9. (×1000) 9. A sporangium in Bi-nucleate stage, showing 2 nucleoles in one of the nuclei. (×320) 10. A sporangium in metaphase of second nuclear division. (×320)

の仁と少数の小染色粒とがある。胞子嚢が次第に大きさを増してくると核はその中央に移動し，核腔内にはうすく染まつた核網か現われ，染色粒は消失する，核網は核膜から遊離し，屢々塊りとなつて仁の近くに集まることがある。この頃には仁は一様に染まらず網目状となり空胞がみられる。この時期には極く稀に2個の仁を有する核や，仁のほかに大きさ並びに形は仁と同じであるが仁よりも染まりがうすく，その内部には仁の如き網目構造かみられない物体か数個みられる核や，又仁のほかに仁と同様に染まり仁の如き網目構造はみられるか形態がやや異なる数個の物体を有する核などか観察された。しかし，エゾヤハズの核分裂前期で観察されたような，仁より小さく，仁とは異なる物体は認められなかつた。核内のうすく染まつた核網上には染色粒が現われ染色粒からやや濃く染まる染色糸か形成され，間もなくこの染色糸は核内の一側部に偏在してループを形成するが，このループはエゾヤハズの核分裂前期にみられた染色糸と比べると染まりがう



Text-figures 11-19. Microphotographs of nuclear division of *Dictyota dichotoma* LAMOUR

11 & 12. Nuclei containing several nucleole-like bodies beside the nucleole, in early prophase of the first nuclear division of tetrasporangia; the same nuclei are shown in Pl. I, fig. 23 and fig. 24 respectively. 13-15. Nuclei in early metaphase of the first nuclear divisions in the tetrasporangia. 16. Side vew of nucleus in anaphase of the first nucler division in a tetrasporangium. 17. Nuceus in early anaphase of the first nuclear division in a tetrasporangium; the same nucleus is shown in Pl. fig. 29. 18. A plantlet growing on the surface of a tetrasporic plant, one large cell of which is in metaphase of somatic nuclear division; the same plantlet is shown in Pl. I. fig. 35. 19. Two cell stage of an abnormally dividing tetraspora ngium, which is shown in Pl. I. fig. 35, the upper cell is in metaphase of somatic nuclear division and the lower is in anaphase. Magnification: 11-18, × 530, 19 × 860.

すく細い。このループは核内に拡がり始めるが益々染まりがうすく且つ細くなり，やがて2本ずつ対をなす染色糸となる。diakinesis 期は観察することは出来なかつた。核分裂中期の始めには32個の染色体が算えられた。核分裂中期では染色体は赤道板に並び紡錘糸がみられるがこの紡錘糸はエゾヤハズでみられたものよりも染まりがうすく，そのため認められない場合が多い。両極には時に小さな中心体らしい小体が認められるが，これは観察されない場合が多かつた。中期には核膜，仁は共に消失するが稀に仁が残存するものがみられた。両極に移動する染色体群の間にはうすく染まつた紡錘糸が認められる。後期では染色体は消失し仁が現われ，核膜が形成され2娘核が形成される。続いて第2回目の分裂が行われるが，やはり32個の染色体が算えられた。この分裂が終ると胞子嚢内に4個の小核が見られ，次いで核膜が生じ，内容は十字状又は三角錘状に分裂して4個の四分胞子が出来上る。四分胞子嚢の分裂には稀に異常分裂がみられた。即ち第1回核分裂が終ると隔膜が形成されて細胞分裂が行われ，四分胞子嚢の内容が直接幼体に生長するのである。Text-figs. 19 は四分胞子嚢から生じた異常発生体を示し，四分胞子嚢での第1回核分裂後に胞子嚢の横軸に平行に隔膜が生じ，胞子嚢は上下2個の細胞に分たれ，下方の細胞は更に2細胞に分裂し，上方の細胞に核分裂が行われているところであり，この核分裂像に32個の染色体が算えられる。四分胞子体上には屢々ア

ミヂグサの幼体がみられるが，その基部は四分胞子体の体細胞と連なり，これらの幼体は四分胞子嚢の内容がそのまま発生して出来たものであることが確められる場合が多い。染色体はこれらの異常発生体の細胞中に於ても32個を算えることが出来た。又，数個細胞から成る幼体で大きさが四分胞子嚢よりも小さく，四分胞子が発芽して生じた体と思われるものの体細胞分裂に於ても核分裂中期の像を観察し得たが，中期の始めに算え得た染色体数は何れも32個であつた。

論　　述

アミヂグサ科植物の四分胞子嚢に於ける核分裂に関しては前述の英国産アミヂグサのほか *Padina pavonia* (CARTER, 1927) についての研究があり，いずれも第1回核分裂前期に仁のほかに spherule なる小体が現われ，又，diakinesis 期の前に特殊な休止期のあることが報告されている。spherule なる小体はエゾヤハズでも多くの場合観察されたが，アミヂグサでは認めることが出来なかつた。WILLIAMS (1904) は *Dictyota dichotoma* で稀に圭角を有する spherule がみられると述べており，(p. 145)，CARTER は *Padina pavonia* で圭角を有する spherule を図示 (Pl. VIII, No. 7, 8.) している。しかし，筆者がエゾヤハズで観察した spherule はすべて小球体であり，多角のものはみられなかつた。筆者がアミヂグサの四分胞子嚢の第1回核分裂前期を多数検鏡したところから推察すると忍路湾産アミヂグサでは spherule なる小体は出現しないか，出現することはあつても，その割合はエゾヤハズに比して極めて少ないものであると考えられる。又，WILLIAMS 並びに CARTER の言う休止期なる stage は，アミヂグサの四分胞子嚢では第1回核分裂で diakinesis 期を観察することができなかつたので果して存在するかどうか確認し得なかつた。しかし，エゾヤハズでは正常な減数分裂が行われ，休止期なる stage は認めることは出来なかつた。染色体数に就いてはエゾヤハズでは猪野の観察と一致し，n＝16であることを確めた。一方，アミヂグサでは四分胞子嚢内での2回の核分裂中期の始めに，又，四分胞子嚢から異常発生した幼体，又は四分胞子の発芽した幼体と思われるものの体細胞分裂で32個の染色体数が算えられた。この染色体の現われた時期及び場所から判断すると，n＝32と考えられるが，この点については今後更に研究を進めて確めたい。エゾヤハズでは中心体は認められなかつたが，これは猪野が四分胞子の発芽による幼体の仮根細胞の中期分裂像で観察した結果と一致する。

以上の観察結果からエゾヤハズとアミヂグサの四分胞子嚢内核分裂には次の如き相違のあることがわかる。即ち，核分裂前期にエゾヤハズでは spherule なる小体があり，核内の一方に偏在する染色糸はよく染まるが，アミヂグサでは spherule なるものは認められず，核内に偏在する染色糸はエゾヤハズよりも細く，染まりがうすい。又，核分裂中期にはエゾヤハズでは核膜は消失し，中心体らしい小体がみられ，且つ，紡錘糸の染まりはエゾヤハズよりもうすい。

摘　　要

1. 北海道忍路湾産エゾヤハズ及びアミヂグサの四分胞子嚢に於ける核分裂を観察し，両種間の核分裂過程の比較を試みた。
2. エゾヤハズの四分胞子嚢の第1回核分裂は正常な減数分裂で，染色体数は n＝16である。
3. 核分裂前期には，エゾヤハズでは spherule がみられ，核内の一側部に形成された染色糸はよく染まるが，アミヂグサでは spherule は認められず，核内の一方に偏在する染色糸はエゾヤハズよりも細く染まり方がうすい。
4. 核分裂中期には，エゾヤハズでは核膜は残存するが，アミヂグサでは消失する。
5. 中心体はエゾヤハズの核にはなく，アミヂグサには存在する。又，紡錘糸はエゾヤハズでは染まりがよく，アミヂグサよりも明瞭に認められる。
6. アミヂグサの染色体は第1回，第2回の核分裂中期の始めに32個を算えた。この数は四分胞子嚢から異

常発生した幼体，又，四分胞子が発芽して生じた幼体と思われるものの体細胞分裂中期にみられた染色体数と一致する。

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PLATE I

Figs. 1–18. Nuclear division of *Dictyopteris divaricata* (Okamura) Okamura

1. Part of a cross section of the thallus, showing tetrasporangia in early stages of their development. (× 520)

2–5. Nuclei in synapsis, in the first nuclear division of tetrasporangia. (×1800)

6–8. Nuclei in diakinesis, in the first nuclear divisions of the tetrasporangia. (×1800)

9. A nucleus in metaphase, in the first nuclear division of a tetrasporangium; the plane of division is as right angle to the longitudinal axis of the sporangium. (×2100)

10 & 11. Nuclei in anaphase, in the first nuclear division of a sporangium; the plane of division is paralell to the longitudinal axis of the sporangium. (×2100)

12. A nucleus in telophase, in the first nuclear division of a tetrasporangium. (×2400)

13. Bi–nucleate stage of a sporangium. (×660)

14. A nucleus in metaphase from the Bi–nucleate stage of a tetrasporangium. (×2100)

15 & 16. Nuclei in anaphase from the Bi–nucleat stage of tetrasporangia. (×2100)

17. Tetra–nucleate stage of a tetrasporangium. (×660)

18. A mature sporangium containing tetra–spores. (×660)

Figs. 19–36. Nuclear division of *Dictyota diclotour* Lamour

19–22. Nuclear divisions prior to the formation of stalk cell of a tetrasporangium. (×1100)

23 & 24. Nuclei in early prophase of the first nuclear division of tetrasporangia, cotaining several nucleole–like bodies in the nuclear cavity. (×2000)

25–28. Nuclei in early metaphase, in the first nuclear division of tetrasporangia. (25–27. × 1800; 28. × 1500)

29. A nucleus in early anaphase, in the first nuclear division of a tetrasporangium; a small centrosome–like dody is seen at one pole. (×1800)

30. Bi–nucleate stage of a tetrasporangium. (×860)

31. Metaphase of the secod nuclear division in a tetrasporangium. (×860)

32. Quadri–nucleate stage of a tetraspornangium. (×860)

33. A mature sporangium with tetraspores. (×860)

34. The same sporangium is shown in Tet–fig. 19. (×920)

35 & 36. Plantlets growing on the surface of tetrasporic plants; the apical cell is in metaphase of the somatic nucler division. (×1500)

北太平洋水域に於けるキタノホッケの仔稚魚*

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Larvae and youngs of the Atka mackerel, *Pleurogrammus monopterygius* (*PALLAS*), in the North Pacific

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Abstract

Larvae and youngs of the Atka mackerel were collected with the fish larva net during the cruises of the "Oshoro Maru" to the Bering Sea and North Pacific in 1955, 1956 and 1957; 34 individuals were obtained, measuring 25.6–44.4 mm in total length, mainly offshore the North Kuriles and Kamchatka during the period from June 10th to July 2nd on these cruises.

Bodily characteristics of these specimens are as follows:

1. Snout bluntly rounded, lower jaw included.
2. No deep emagination is found between the spinous and soft rayed portion of the dorsal fin throughout these specimens.
3. Pectoral fin is rather small and round in shape.
4. The caudal fin emarginated rather deeply in posterior margin, and has no vertical bar.
5. A pair of supraocular flaps appears in the stage of ca. 26–27 mm in total length for the first time.
6. Color is deep blue-green, and black spots in a line are not found on the lower side of body; all fins are colorless.

緒　　言

従来アイナメ科の幼稚魚に関する研究は多くの研究者に依つて行われ，特に *Hexagrammos otakii* JORDAN & STARKS アイナメに就いては大島・中村('44)及び山本・西岡('48)等に依つて生活史及び初期発生等に就いて述べられ，又中村('36)に依つて後期稚魚及び幼魚に就いて報告されて居るが，其の他のアイナメ属のものに就いては未だ報告を見ない。又ホッケ属のものに就いては *Pleurogrammus azonus* JORDAN & METZ ホッケに就いて北水試('53)に依る研究の外，Abe('51)及び蒲原('50)に依り其の幼稚魚形態に就いて詳しく述べられて居るが，*P. monopterygius* (PALLAS) キタノホッケの幼稚魚に関しては未だ研究報告が無い。

筆者は1955～1957年の主として6～8月の間に北海道大学練習船おしょろ丸に依つて採集された資料の内 *P. monopterygius* キタノホッケの幼稚魚の形態等に関する観察結果を此処に報告する。

猶本論に入るに先立つて，本研究の機会を与えられ，終始御指導を賜つた，本学佐藤信一教授に深甚なる謝意を捧げ，又採集に当つて絶大なる御協力を戴いた，おしょろ丸船長藤井武治氏以下乗組員の諸氏並びに各年に於ける北太平洋海洋観測団員の諸氏に感謝の意を表する。猶対照参考として日本海より得られた *P. azonus* ホッケの仔稚魚標本を貸与された北海道区水産研究所竹内勇技官に厚く謝意を表する。

材　　料

標本は 1955年6～8月，1956年7～8月及び 1957年6～7月の間，おしょろ丸に依り口径 130cm，長さ 450cm（綟子網300cm + GG54. 150cm）の稚魚ネットを平均2節で10分間曳網して採集した。標本は採集後

* おしよろ丸北洋調査報告 No. 10

直ちにホルマリン液で固定した。

1955年及び1956年の全採集位置並びに採集資料の概要は海洋調査漁業試験要報第1号に報告した。（海洋漁業要報．第1号（1957),北大水産）又1957年の概要は同じく第2号に報告の予定である。猶本種の採集された水域を中心として採集点を示すと図の如くである。（Fig. 1.）即ち1956年の採集では本種は全く得られて居ない。又採集された水域は主として北千島及びカムチャッカ半島寄りの水域である。第1表にも見られる様に，1955年に3採集点で6尾，又1957年には7採集点で28尾，計34尾が3年間の採集で蒐められた。又採集された期間は6月10日から7月2日の間である。

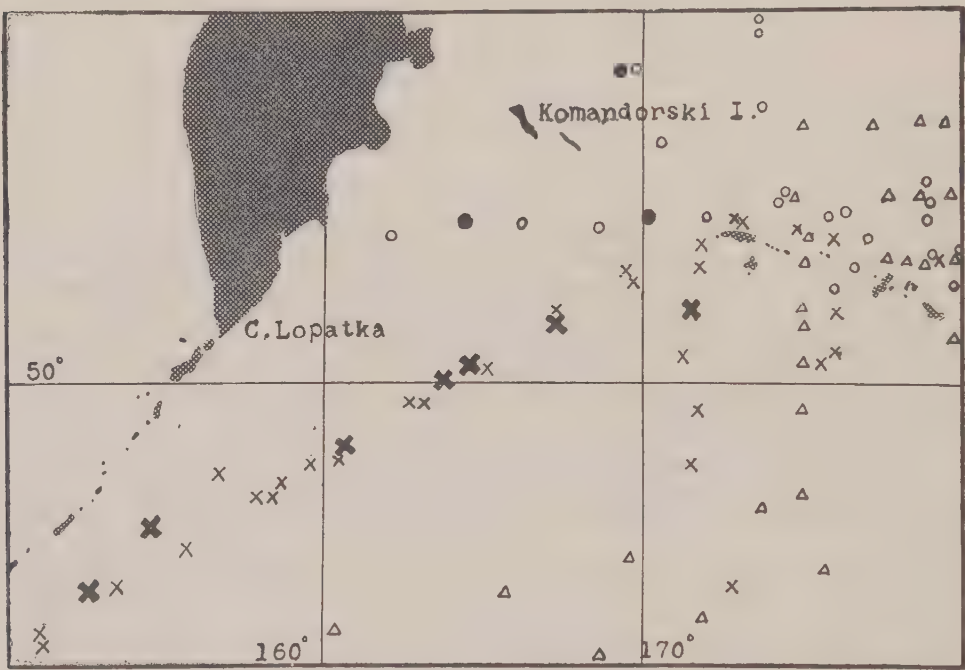


Fig. 1. Stations and collection-sites of larvae and youngs of the Atka mackerel, *Pleurogrammus monopterygius* (PALLAS), by "Oshoro Maru" in 1955, 1956 and 1957
○ stations in 1955 ● collection-sites of the Atka mackerel larvae and youngs, in 1955
△ stations in 1956
× stations in 1957 **X** collection-sites of the Atka mackerel larvae and youngs, in 1957

仔稚魚の形態

蒐められた標本中より代表的なものの形態に就いて以下に記述する。（Fig. 2. 参照）

"A" 全長25.7mm；体長22.1mm；頭長5.2mm；吻端より肛門迄の距離10.3mm

吻端は円い。各鰭は何れも未完成で，背鰭に43条，臀鰭に25条，胸鰭に23条が数えられるが何れも定数には達して居ない。腹鰭は認められるが鰭条は発現して居ない。尾鰭の後縁は截断型に近い半円型である。又尾索が明瞭に認められる。

体は全体に青緑色で，頭部並びに背側に青色が濃い。体側に黒褐色小点が密集して僅かに2列の帯状を為す。背鰭基底に沿つて僅かに小黒点がほゞ一列に点在する。両眼間隔の稍々後方に瞳孔大の黒斑がある。鰭は何れも無色である。（Fig. 2，A）（標本番号459,L）

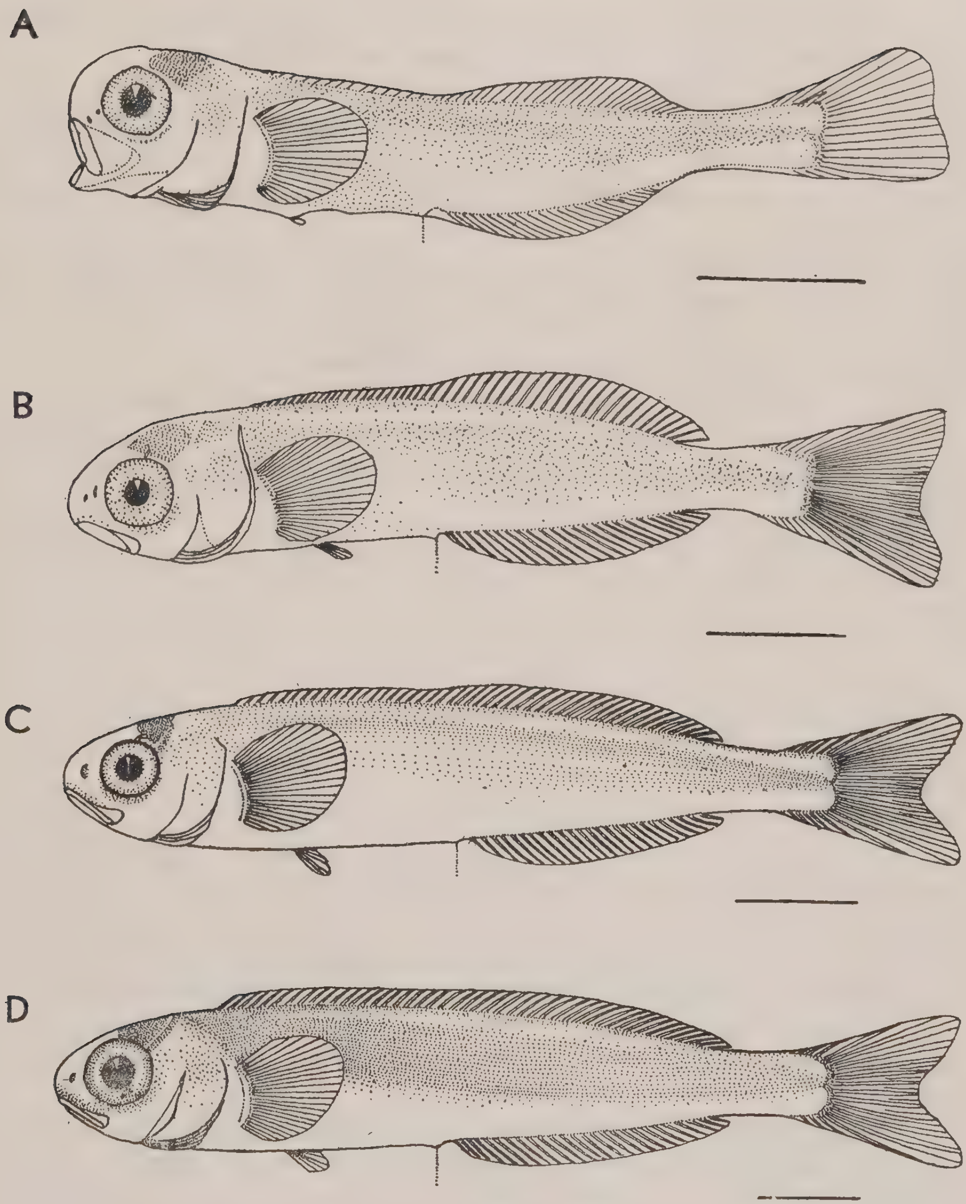


Fig. 2. Larvae and youngs of the Atka mackerel, *Pleurogrammus monopterygius* PALLAS
A：25.7mm in total length；B：30.7mm；C：36.9mm；D：44.4mm

"B" 全長30.7mm；体長25.8mm；頭長6.3mm；吻端より肛門迄の距離13.1mm

吻端は円く，下顎は上顎より短い。眼窩上縁に皮質突起がある。鰭条は何れも完成され，背鰭に48条，臀鰭に25条，胸鰭に25条，又腹鰭に6条が数えられる。背鰭の前半は他の鰭に比し発達弱く低い。尾鰭の後縁は稍々深く2叉し，中部の約4条が末端に於いて極く僅かに分枝するが不明瞭である。

体は全体に青緑色で，頭部及び背側に特に青色が濃く，体側中央に沿つて黒褐色小点が密集して帯状を為

すが，中央線部が僅かに疎で前半に於いては不明瞭乍ら2列となる。両眼間隔の稍々後方に一対の瞳孔大の黒斑がある。各鰭は何れも無色である。(Fig. 2, B)(標本番号459, D)

〃C〃　全長36.9mm；体長31.3mm；頭長8.1mm；吻端より肛門迄の距離16.1mm

吻端は円く，下顎は上顎より短い。眼窩上縁に皮質突起が明瞭に認められる。鱗並びに側線は全く認められない。各鰭条は完成し，背鰭に47条，臀鰭に25条，胸鰭に25条並びに腹鰭に6条が数えられる。背鰭の前半は低いが後半との間に凹部は無い。尾鰭の後縁は稍々深く2叉し，中部の約5条が末端に於いて僅かに分枝するが不明瞭である。

体は全体に青緑色で，背側及び頭頂部では特に青色が濃い。体側稍々中央に黒褐色小点が密集して巾広い帯状となつて縦走する。両眼間隔の稍々後方に一対の瞳孔大の黒斑がある。各鰭は何れも無色である。(Fig. 2, C)(標本番号7)

〃D〃　全長44.4mm；体長37.8mm；頭長8.8mm；吻端より肛門迄の距離18.7mm

吻端は円く，下顎は上顎より短い。眼窩の上縁に皮質突起が微小乍ら認められる。鱗並びに側線は全く認められない。各鰭条は完成し良く発達する。即ち背鰭に46条，臀鰭に26条，胸鰭に25条並びに腹鰭に6条が数えられる。背鰭に凹部は無い。尾鰭の後縁は稍々深く2叉し，中部の約5条が末端に於いて僅かに分枝する。

体は全体に青緑色で背部では特に青色が濃く，体側の稍々中央に黒褐色小点が密集して巾広い帯状となつて縦走する。両眼間隔の稍々後方に一対の瞳孔大の黒斑がある。各鰭は何れも無色である。(Fig. 2, D)(標本番号4)

考　察

次に蒐められた材料を通じて，本種の仔稚魚期に於ける体各部の形成状態其の他に就いて考察すると以下の如くである。(Table 1. 参照)

〃**頭長並びに肛門の位置**〃

頭長は表に見られる様に，全長の19.8〜23.4%で，本資料を通じては特に其の成長による変異は認められない。又肛門の位置に就いて，吻端より肛門迄の距離（s〜a）を以て表現した，即ち資料を通じて全長の39.9〜47.4%で，数値にはかなり差があるが本資料では肛門は全長の中央より稍々前位にある事が知られる。

〃**鰭**〃

背鰭棘条部は全長25mm前後では極めて低く，鰭条も辛うじて計測出来る程度であるが，30mm前後で鰭条はほゞ定数に達する。然し他の鰭に比し発達は弱く低い。40mm前後で背鰭軟条部と其の高さはほゞ等しくなる。

背鰭軟条部は比較的早くから鰭条も完成され，臀鰭と共に全長約27mmで定数に達し，30mm前後で完成される。

胸鰭は背鰭軟条部，臀鰭等と共に，比較的早くから鰭条が定数に達し，30mm前後で完成される。

腹鰭は鰭条の発現は遅く，25mm前後のものでは鰭条は発現せず，27mm前後で発現し始めて，間も無く定数に達する。

尾鰭は本資料中初期のものでは後縁は截断型に近い半円型であるが，次第に尾叉は深くなる。又尾索は初期のもので明瞭に認められるが間も無く消失し，全長約30mm以上のものには全く認められない。

〃**皮質突起**〃

眼窩上縁に一対の皮質突起が，全長26〜27mm前後で認められる様になり，30mm前後では良く発達する。

〃**体色**〃

体は全体に青緑色で，顕著な特徴を示す。特に頭部及び背側に青色が濃い。又体側に黒褐色小点が密集して帯状を為すが，初期のものでは僅かに2本の帯状となつて縦走し，全長約30mmで巾広く一本となる。両

Table 1. Measurements and counts of larvae and youngs of the Atka mackerel, *Pleurogrammus monopterygius* (PALLAS) ( ): unfinished form

Date	St. No.	T.L.mm	B.L.mm	H.L. in T.L. %	s-a in T.L. %	D.	A.	P.	V.	flap
'57, VI, 22	17	25.6	22.0	21.4	41.0	(41)	(25)	(23)	( )	
'57, VI, 21	16	25.7	22.1	20.2	40.0	(43)	(25)	(23)	( )	
〃	〃	26.2	22.3	21.0	40.8	(48)	(25)	(23)	( )	
'57, VI, 22	18	26.5	22.1	23.4	43.4	47	24	(23)	(5)	
'57, VI, 10	5	26.6	23.2	21.4	42.8	(27)	(26)	(20)	( )	
'57, VI, 21	16	26.8	23.0	21.2	39.9	(45)	(24)	(23)	(2)	1
〃	〃	26.8	23.0	21.6	42.5	(45)	(25)	(23)	(5)	
'57, VI, 22	17	27.2	23.1	21.3	40.7	(45)	(25)	(23)	( )	1
'57, VI, 20	14	27.8	23.8	20.5	42.1	(44)	(25)	(23)	(5)	
'57, VI, 21	16	28.3	24.2	21.9	42.4	48	25	(23)	6	1
'57, VI, 11	6	29.2	25.3	21.9	43.2	(44)	(23)	(24)	6	
'57, VI, 21	16	29.5	25.1	21.0	42.0	48	26	24	6	1
'57, VI, 22	17	30.3	25.7	21.1	42.9	(47)	25	25	6	
'57, VI, 21	16	30.7	25.8	20.5	42.6	48	25	25	6	1
'57, VI, 26	21	31.0	26.3	21.9	47.4	47	25	(23)	6	1
'57, VI, 22	17	31.1	26.7	21.5	43.1	49	25	23	6	1
'57, VI, 26	21	31.2	26.2	22.4	43.9	47	24	(24)	6	
'57, VI, 20	14	31.3	26.7	20.4	41.5	48	26	(24)	6	
'57, VI, 21	16	31.8	27.1	20.4	44.0	47	25	24	6	1
〃	〃	32.6	27.5	21.1	43.2	49	25	25	6	1
'57, VI, 20	14	32.6	27.8	20.5	42.3	46	23	25	6	1
'57, VI, 22	17	33.3	28.2	21.3	43.8	48	25	25	6	1
'55, VI, 30	5	34.1	29.0	20.8	44.0	46	27	27	6	1
'55, VII, 2	8	35.0	30.0	20.5	44.0	46	25	25	6	
'57, VI, 21	16	35.1	29.7	20.8	43.3	48	24	24	6	1
'55, VI, 30	5	35.2	29.6	22.1	43.4	47	26	25	6	1
'57, VI, 21	16	35.9	30.7	20.9	42.3	51	26	25	6	1
'57, VI, 10	5	36.5	31.0	21.9	45.2	46	27	25	6	1
'57, VI, 26	21	36.5	31.1	22.2	42.7	48	26	26	6	1
〃	〃	36.6	30.5	21.6	45.3	49	26	24	6	1
'55, VI, 30	5	36.9	31.3	21.9	43.6	47	25	25	6	1
'57, VI, 26	21	39.1	32.7	20.2	44.7	47	24	26	6	1
'55, VI, 28	2	41.4	34.7	22.2	45.9	48	25	28	6	1
'55, VII, 2	8	44.4	37.8	19.8	42.1	46	26	25	6	1

眼間隔の稍々後方にほゞ瞳孔大の黒斑が一対ある。

〃他種との比較〃

本種と同属に属する *P. azonus* JORDAN & METZ ホッケと，其の仔稚魚期に於ける形態等に就いて比較するに，北部日本海より得られた資料に就いて見ると，全長20mm前後で背鰭，臀鰭及び胸鰭に鰭条が発現して居り，胸鰭はほゞ定数に達して居る。又25mm前後で背鰭及び臀鰭も定数に達し，種間の差異がほゞ明

らかになる。其の他色彩等に関しては特に差異は認められない。猶此の日本海より得られたホッケの仔稚魚は3月中旬から4月上旬に採集されたもので全長10.5～45.0mmであり，本報告のキタノホッケの仔稚魚は6月初旬から7月初旬に採集された全長25.6～44.4mmのものである。又 Abe ('51) は日本海から3月下旬に得られた全長45mm, 63mm又体長18.0mm, 21.3mm, 26.7mmのホッケの仔稚魚に就いて述べて居り，更に又蒲原 ('50)は北海道太平洋沿岸日高地方に於ける，6月中旬体長48～140mm，7月上旬体長100～150mm及び同下旬体長130～160mm等の資料に基いて報告して居る。即ち以上の事から両種の発生時期には相当差がある事が推察される。

次に北洋水域に於いて本種と同時に採集される *Hexagrammos decagrammus* (Pallas) 及び *H. stelleri* Tilesius エゾアイナメの仔稚魚との差異並びに本種の特徴を述べると下記の如くである。

1. 吻は円く，下顎は上顎より短い。
2. 背鰭は其の発達過程に於いても背鰭棘条部及び背鰭軟条部の間に凹部は無い。*H. decagrammus* 及び *H. stelleri* では凹部が明瞭である。
3. 胸鰭条の定数は25～26で，*P. azonus* より稍々多く， *H. decagrammus* 及び *H. stelleri* の20前後より遙かに多い。又胸鰭は比較的小さく，ほゞ円型である。*H. decagrammus* 及び *H. stelleri* では比較的大きく，先端は稍々尖る。
4. 尾鰭後縁の切れ込みは比較的深く，横縞が無い。*H. decagrammus* 及び *H. stelleri* ではむしろ截断型に近く，横縞がある。
5. 皮質突起は眼窩上縁に一対ある。*H. stelleri* では同じく一対あるが，*H. decagrammus* では更に項部に一対，計2対存在する。
6. 体色は青緑色が特に濃く，体側中央と臀鰭基底のほゞ中間に一列に並ぶ黒点が無い。又此の資料を通じては吻端附近に黒褐色素は殆んど見られない。各鰭は何れも無色である。 *H. decagrammus* 及び *H. stelleri* では体は同じく青緑色であるが，褐色素がかなり見られる。又体側中央線下に黒色点が一列に並ぶ。又吻端に相当初期より褐色素が現はれる。*H. decagrammus* では鰭は無色であるが，*H. stelleri* では鰭は何れも淡褐色である。

要　　　約

北太平洋水域に於いて，1955年6～8月，1956年7～8月及び1957年6～7月の3ヶ年間に亘る，おしょろ丸に依る稚魚ネット採集の結果，キタノホッケの仔稚魚は主として北千島及びカムチャッカ半島寄りの水域で6月10日から7月2日の間に得られた。猶形態的に下記の特徴を有する事が知られた。

1. 吻は円く，下顎は上顎より短い。
2. 背鰭は其の発達過程に於いても，背鰭棘条部及び背鰭軟条部の間に凹部は無い。
3. 胸鰭は比較的小さく，ほゞ円型である。
4. 尾鰭後縁の切れ込みは比較的深く，横縞が無い。
5. 皮質突起が眼窩上縁に一対，全長26～27mm前後で発現する。
6. 体色は青緑色が特に濃く，体側中央と臀鰭基底のほゞ中間に一列に並ぶ黒点が無い。各鰭は何れも無色である。

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北太平洋に於けるベニサケの年令と系統について

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A Note on the Age and Race of the Red Salmon in the North Pacific Ocean

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Abstract

It has been known that the populations of the red salmon which migrate for spawning are most commonly composed of the 4_2, 5_2, 5_3 and 6_3 age-groups. In the populations of the red salmon of south-eastern Alaska and British Columbia, the 6_3 age-class fish are conspicuously few in number, while in the Aleutian area, the 6_3 age-group is predominant. Also there can be found a tendency for the percentage of the 6_3 age-class fish to become lower towards the eastern part of that area.

Although the salmon of Bristol Bay are similar, in respect of the age composition to those of the Aleutian area, it is found that the former differs in radius and growth pattern of scales from the latter, but further investigation on this point must be carried out.

It is recognized that the red salmon caught along Asiatic coasts and in the Aleutian area are markedly smaller both in length and in weight than those of North American coasts, even for the 6_3 age-group.

Therefore, it is most reasonable to assume that the populations of the red salmon caught in the Aleutian area may not be of North American origin. This supposition seems to be strengthened by another fact that in the latter half of the fishing season, the percentage of the 6_3 age-class fish decreases in the north-western part of the Aleutian area, while it increases in the Okhotsk Sea. In other words, it seems evident that the most of the populations of the red salmon in the Aleutian area migrate westwards as the season advances.

北アメリカ大陸沿岸及び河川に於けるベニサケの年齢に関する研究は Gilbert (1914) によつて始められ，その後，永年継続の調査として他の研究者によつて受けつがれ，現在 Foskett (1951-1955) によつて担当されている。この研究の結果として，各地域のベニサケの魚群はその年齢組成に於て夫々連年同じような特色があり，いわゆる Gilbert•Rich の方式* (Gilbert & Rich, 1927) で表示すれば 4_2, 5_2, 5_3 及び 6_3 と言う四つの年齢群が組成の主体をなしている事が判つた。又 Gilbert (1922) は北西アラスカ方面のベニサケには高年齢のもの多く，カナダ及びアメリカ西岸のものには若年齢のものが多い事を報告している。

一方，アジア大陸沿岸並に北洋のベニサケの年齢については横山・花井(1931)，横山・川上(1932)，並に田口 (1942,1948) の報告があるが，これらに於てはウロコの中心部に於ける年輪の判読の基準が明確にされていないので，直ちにこれらを引用する事は困難のようである。その後，久保・山平(1953; 1955) は北洋のベニサケの年齢に関する調査結果を岡田の方式** (岡田,1939) によつて表示し，北アメリカ大陸方面のベニサケと比較出来る資料を発表した。

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* スモルトとして降海した年次を下付数字として明けの年齢に附記する方式である。例えば 6_3 は 3 年目の春に降海し，生活の第 6 年目にある魚の年令を表す。

** 淡水生活時代の年輪数を記号Fの下付数字で，海洋生活時代の年輪数を記号Sの下付数字で表すもので，直ちに Gilbert•Rich の方式に転換表示出来る。例えば F_2-S_3 は 6_3 である。

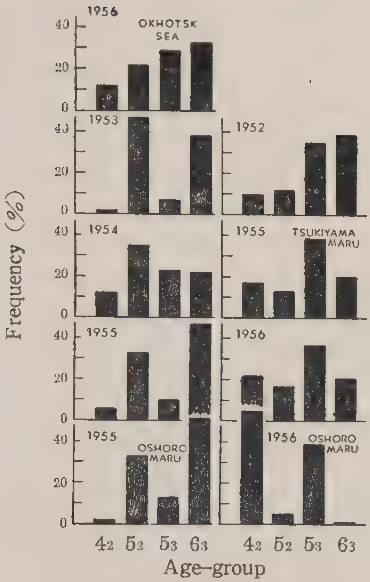
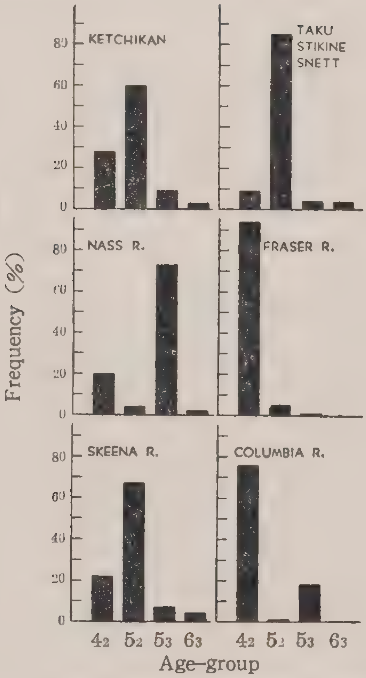
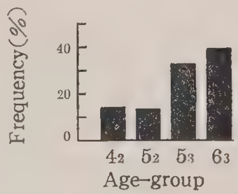


Fig. 1 Histogram showing age composition of red salmon in the Aleutian area and the Okhotsk Sea



A- South-eastern Alaska and British Columbia



B- Bristol Bay

Fig. 2 Histogram showing age composition of red salmon in rivers or coasts of Northern America, in 1955

筆者はこれ迄の諸報告及び最近の調査資料の中で，主として年齢組成に関するものについて考察し，ベニサケの系統に関する一二の知見を得たので玆に報告する。

本研究に於て用いられた資料の一部は北太平洋三国漁業条約に基き水産庁が行つている調査によつて得られたものであり，研究の機会を与えられ，且，未発表資料の引用を許された水産庁調査研究部長藤永元作氏に深く謝意を表する。又，現地に於ける材料採集，魚体測定に力を尽された水産庁山平喜一郎氏他の調査官各位，各会社母船，調査船乗組の調査担当者各位並に北大水産学部おしょろ丸乗組の調査員小林喜雄，久新健一郎の両氏，種々教示を賜つた笠原昊，平野義見，田口喜三郎の諸氏，及び原稿を校閲され忠言を賜つた山本喜一郎教授に深謝の意を表する。

年齢組成について

1952—'56年の北洋のベニサケの年齢組成を示せば第1図*の通りとなる。又第2図，A，B**には1955年北アメリカ大陸の沿岸並に河川に来游泝河したベニサケの年齢組成を示した。

前記の通り，産卵洄游時のベニサケの魚群を構成する主要年齢群は 4_2，5_2，5_3及び6_3の4群で，その他に属する魚は極めて少い故，こゝでは便宜上それらを除外して表示した。

*久保・山平（1953;1955），田口（1955），水産庁（1956），北大水産（1957）及び水産庁（1956年の分，久保担当未発表）より引用。

**この基礎資料は漁業国際委員会の協約に基き相互に交換されたウロコの標本について筆者が観察して得たもの。

第1図に於て全体を通じて認め得る特徴は6_3年群が卓越するか，若しくはそれに近く大きい組成を示す事である。それに次で5_2，5_3年の組成が大きいが，6_3年群及び5_2年群の少い場合には逆に5_3年群が多く現れる。一般に漁場位置が東側に偏れば6_3年群が少くなる傾向があるが，しかしこのような場合にもそれの組成が，20%を下らない事が大部分のようである（第1図右側系列）。但し1956年のおしょろ丸の材料に於ては6_3年群が極めて少いが，これはその採集場所が著しくアリューシャン海域の東北方に偏している事と関係があるかも知れない。又，漁場位置が西側に偏る時には6_3年群の卓越性が特に著しく，同時に5_2年群も割合に多く現れる（第1図左側系列）。尙，比較の為にオコック海に於けるベニサケの年齢組成を示した。この場合には 5_2，5_3両年群共に比較的多く現れるが，6_3年群の卓越する事はアリューシャン海域のものと同じである。

他方，北アメリカ大陸沿岸及び河川に於けるベニサケの年齢組成は第2図に示される通り，Bristol湾の場合を除いては何れの場所に於ても6_3年群の組成が極めて小さい。このような事は亦 Foskett (1955) によつても示されている。即ち，カナダ西岸方面のベニサケではこのような年齢組成は，Gilbertの調査開始以来，毎年同じように認められていて，河川及び地区によつて夫々かなり異つた特徴を示すにも拘らず，6_3年群の組成の小さい事では共通している。又，Gilbert と Rich (1927) によれば中部アラスカの Karluk 河のベニサケでは特別の年にはかなり多くの6_3年群が現れているが，一般にはそれの組成が小さい傾向にある。Bristol湾のベニサケの年齢組成については最近には，まとまつた報告は見れらないが，Gilbert (1923) によれば，Egegik, Naknek 及び Kvichak 河では6_3年群が夫々46，35及び27%出現している。これと筆者の観察結果とを併せ考えるならば Bristol 湾に於けるベニサケには例外的に6_3年群の多く現われる事は否定出来ないようである。しかしそのような特別の場合を除けば，北アメリカ大陸沿岸のベニサケの年齢は，全般的に見てカムチャッカ沿岸及びアリューシャン海域のそれとは対称的に6_3年群の組成が小さいと言えよう。

体長及び体重について

久保・山平 (1955) によれば，1953年，175°E以東のアリューシャン海域に於て商業船団によつて漁獲されたベニサケは一般に小型であり，平均体長56.1cm，平均体重2.2kgである。しかもこの魚群はその大部分が6_3及び5_2年群に属するものから成つている（共に海洋生活4年目のもの）。水産庁 (1956) によれば，1955年，アリューシャン海域に於て商業船団によつて漁獲されたベニサケの年齢組成に於ては大型群である6_3年群，5_2年群が著しく多く現れ，その反面小型群である5_3，4_2年群は極めて少いが，その体長分布のモードは55cm，体重分布のモードは2.0kgである。又，田口 (1955) によれば，北千島に比較的近接したアリューシャン西部海域に於て1955年7月に漁獲された6_3年群ベニサケの体長は，雌では平均56.41cm，雄では平均58.43cmである。

一方，Foskett (1955) によつてまとめられたカナダ西岸各地のベニサケの体長，体重に関する連年の調査結果を要約引用すれば下記の通りで，アリューシャン海域のベニサケよりも大きい値を示している。

District	1952 Average length (cm)	1952 Average weight (kg)	1953 Average length (cm)	1953 Average weight (kg)	1954 Average length (cm)	1954 Average weight (kg)
Nass River	62.2	2.9	63.0	2.99	62.4	3.08
Skeena R.	60.1	2.63	61.1	2.9	61.7	2.99
Rivers Inlet	60.1	2.99	58,0	2.54	58.9	2.81
Smith I.	63.4	3.26	58.2	2.67	59.2	2.67

又，Gilbert と Rich (1927) によれば，中部アラスカの Karluk 河に泝上するベニサケの体長並に体重は1926年6月の6_3年群に於ては，平均体長は雌では61.7cm，雄では63.7cm，平均体重は雌では2.49kg，雄では2.71kgである。6_3年群より魚体の小さい5_3年群の1916—1926年の間の平均体長は雄，59.9cm，雄，63.2cm

であり，この場合も亦，アリューシャン海域のベニサケより大型である。

従つてアリューシャン海域のベニサケは体長，体重に於てアラスカ並にカナダ西岸のベニサケよりも小さい事は明である。しかし乍ら Koo (1955) によれば Bristol 湾の西側河川に泝上するベニサケの体長は雌では57cm，雄では 60cm の辺に分布のモードがあるようである。6_3年群の組成の大きい所の同湾の東側河川のものの体成長に関する資料が得られないので明確な比較は出来ないが， Bristol 湾のベニサケはアラスカ沿岸の他の場所のベニサケのようにアリューシャン海域のベニサケよりも大きいと言い切る事は困難のようである。

考　　　察

アリューシャン海域に於て漁獲されるベニサケの年齢組成に於ては，特別の場合を除いて6_3年群が連年同じように卓越し，亦5_2年群も比較的多く現れる。これらは共に成熟年齢に達した高年魚群（海洋生活 4 年目と考えられるが，体長，体重に於てはそれ程大きくはない。このような傾向は比較的東部の海域（175° E よりも東側）に於ても認められる。

一方，中部及び東南部アラスカ並にカナダ西岸の沿岸に産卵のため来游するベニサケ魚群では6_3年群が極

Table 1　Change of age composition of red salmon in the Aleutian area in keeping with the advance of the fishing season, in 1956; number in parentheses indicates percentage; from Japanese Fisheries Agency (unpublished data)

Fishing ground	Season	4_2	5_2	5_3	6_3	Total
North-western North Pacific	May	88	203	170	191 (29)	652 (100)
	June	204	163	325	188 (21)	880 (100)
	July	153	38	275	108 (19)	574 (100)
	Aug.	100	17	164	42 (13)	323 (100)
Okhotsk Sea	June	12	34	29	19 (20)	94 (100)
	July	32	47	79	103 (40)	261 (100)
	Aug.	7	7	10	10 (30)	34 (100)

Probability of seasonal change of age composition proved by χ^2-test

North-western North Pacific:

	May	Jnne
5_3	170(47)	325(63)
6_3	191(53)	188(37)
	(100)	(100)

$P<10^{-5}$

	June	July
5_3	325(63)	275(72)
6_3	188(37)	108(28)
	(100)	(100)

$P<10^{-3}\times 5$

	July	Aug.
5_3	275(72)	164(80)
6_3	108(28)	42(20)
	(100)	(100)

$P<10^{-6}$

	May & June	July & Aug.
5_3	495(57)	439(75)
6_3	379(43)	150(25)
	(100)	(100)

$P<10^{-6}$

Okhotsk Sea:

	June	July & Aug.
5_3	29(60)	89(44)
6_3	19(40)	113(56)
	(100)	(100)

$P<0.03$

めて少く，しかもその体長，体重はアリューシャン海域のそれよりも大きい値を示している。

従つて現在，日本の漁船の操業している範囲のアリューシャン海域のベニサケは，少くとも中部及び東南部アラスカ並にカナダ西岸のものとは別の系統に属するものであろうと推測される。但し，Bristol湾のベニサケは年齢組成並に体長，体重に於て，アリューシャン海域のそれとの間に大きい差異が見られるに至つておらず，又，Bristol湾に来游すべきベニサケの魚群の一部が或る季節にはかなり西方の海域，特にベーリング海の中央部迄洄游進出することも想像される。従つて両者の系統的差異の問題については現在の段階では言及し難い。

しかし筆者は別の研究（未発表）に於て6_3年群ベニサケのウロコの大さ，成長形式に関して両者の間の差異を認めており，更に今後の調査によりその系統的差異を論ずる予定である。

アリューシャン海域で漁獲されるベニサケの大部分及びカナダ西岸の系統のものではなくアジア大陸の系統に属するものであろうと言う推論は更に次の事柄により強調される。即ちアリューシャン海域のベニサケの魚群の年齢組成には季節的変化が認められ，それにより魚群の季節的移動を推知出来る。第1表の左側を見て判る通り1956年には，6_3年群の組成はアリューシャン海域では月を追つて減少するが，逆にオコック海ではそれが増大する傾向がある。各年齢群の中で6_3年群と共に多く出現する所の5_3年群と6_3年群の出現の比率をχ^2－検定によつて吟味すれば，第1表の右側に示される通り，それぞれの季節による変動はかなり顕著である。アリューシャン海域では，漁期の初には5_3年群が少く，その後次第に増加して行くのに対して，6_3年群は初に多く，終末に至つて少くなる。この相関関係は漁期を前半，後半に2区分して比較すれば一層明白である。一方，オコック海では漁期の前半には6_3年群はそれ程多くないが，後半には著く多く現れる。換言すれば，アリューシャン海域の6_3年群を主体とするベニサケ魚群は漁期末に他に移動し去り，逆にカムチャッカ半島沿岸及びオコック海には6_3年群を主体とするベニサケ魚群が他より来游すると言う事が出来る。

先に第1表について説明した通り，漁場が西方に偏つた年には特に6_3年群の組成が大きい傾向が認められる事と併せ考えるならば，それらの魚群は季節の推移と共に西方に移動し，その一部はカムチャッカ半島の東側河川に溯上するが，他の大部分は西方に回り，オコック海に現れるものであろうと推測される。

摘　　要

アリューシャン海域で漁獲されるベニサケの年齢組成に於て6_3年群の比率が極めて大きい。特に西部海域ではこの傾向が著しい。

北アメリカ大陸の沿岸及び河川で漁獲されるベニサケの年齢組成に於ては6_3年群の比率は比較的小さい。特にアラスカ東南部並にカナダ西岸のものに於ては，その比率は極めて小さい。

6_3年群ベニサケの組成は漁期の後半にアリューシャン海域では小さくなり，逆にオコック海ではそれが増大する。このことから6_3年群の組成の大きいベニサケの魚群は季節の推移と共にアリューシャン海域よりアジア大陸沿岸に向つて移動するものと推測される。

アリューシャン海域で漁獲されるベニサケの大部分はその年齢組成並にそれの季節及び海域による変異から見て，少くともアラスカ及びカナダ西岸の系統のものでない事が推測される。

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サメ肉腐敗の微生物化学的研究

第4報　尿素分解菌の尿素分解能力に及ぼす温度の影響について

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Studies in the Bacteriological Chemistry of Shark Muscle Spoilage

IV. On the effects of temperature upon the urea decomposing ability of the urea-splitting bacteria isolated from spoiling shark muscle

Takahisa KIMURA

Abstract

In the present work, the effect of temperature upon the ability to decompose urea possessed by K-10 strain, which has stronger urease activity than the other four isolated urea-splitting bacteria[1] was observed.

The results can be summarized as follows;

1) The multiplication of this strain showed maximum at about 35°C and much decreased at over 45°C or under 25°C.
2) The optimum temperature of the urease activity of this strain laid at about 60°C, and became remarkably inactive at over 60°C but it showed comparatively higher activity at such lower temperature as those near 0°C.
3) It was inferred from the results obtained as 1) and 2), that the optimum to promote the urea decomposing ability of this strain laid at about 40°C.
4) The urea was decomposed comparative quickly even at such a low temperature as −5°C by the living cell of this strain, which has already grown and multiplied in above cultivation.
5) It is inferred from 4), that urea decomposition of shark muscle in refrigeration depend decidedly upom the freshness of the raw materials.

著者は前報に[2]おいて，鮫肉より分離した尿素分解菌 K-10 株の尿素分解能に及ぼす水素イオン濃度の影響について報告し，その至適水素イオン濃度は pH 7.9 前後にあることから，鮫肉の鮮度保持，特に尿素の分解を阻止するためには可及的に新鮮なときにおいて，換言するならばでき得る限り鮫肉の pH が酸性領域にある間に，何等かの手段によつて尿素分解菌の尿素分解能力を抑制するならばより効果的であらうと推論した。本報においては該菌の尿素分解能力，すなわち発育増殖及び Urease 活性に及ぼす温度の影響，特に魚類の鮮度保持の一重要方法である低温（冷蔵）保存法に関連して，低温度における影響についても実験を試み，若干の知見を得たのでこゝに報告する。

I　K-10株菌の発育に及ぼす温度の影響

a）実験方法

前報[2]に準じて比色用試験管中に分注したペプトン水に一定量の供試菌を接種後（30°C，24時間培養のペプトン水培養液 0.1cc）20°C～45°C の各温度で培養し，光電比濁法により一定時間隔に発育の程度を測定した。

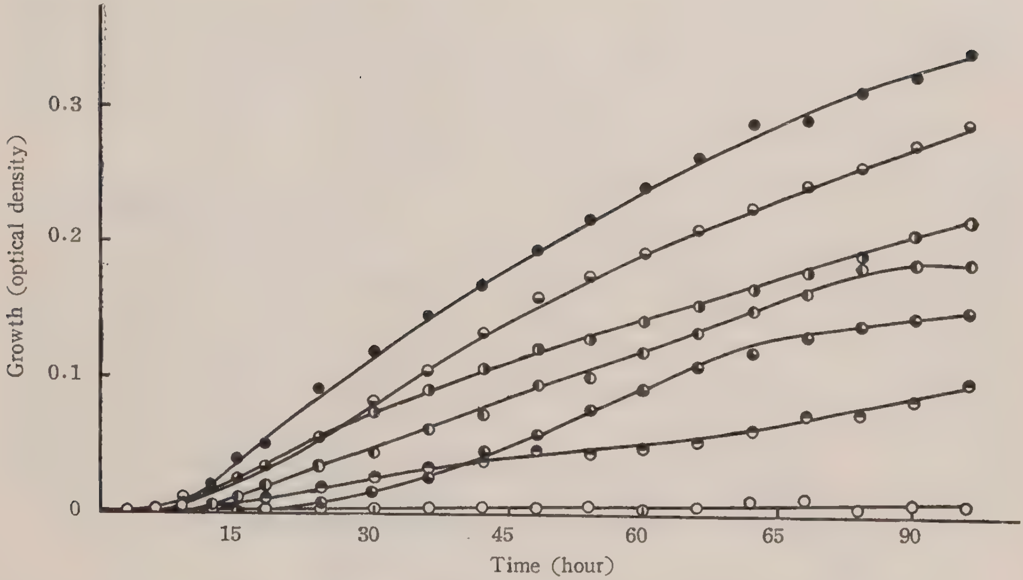


Fig. 1 Effect of temperature on the growth of K-10 strain
○ 0°C ◐ 20°C ◐ 25°C ◑ 30°C ● 35°C ◒ 40°C ◓ 45°C

b） 実験結果

以上のやうな方法により，K-10株菌の発育に及ぼす温度の影響を観察した結果は Fig 1 に示す通りである。すなわちいづれの温度においても約10時間の lag phase の後増殖を開始し，35°C で最も旺盛な発育が見られ，次いで40°C，30°C，25°C，20°C，45°C，の順に発育度が低下し，45°C 以上の高温及び25°C 以下の低温における発育はきわめて緩慢であつた。

II K-10株菌の Urease 活性と温度の関係

a） 実験方法

i 酵素液の調製……前報[2] 同様の方法により調製した凍結乾燥菌体浮游液を酵素液として用いた。

ii 酵素活性の測定……同じく前報[2] に準じ，上記酵素液 0.5cc 及び pH 7.9 燐酸塩緩衝液1.0cc，終末 2 %の基質尿素液 0.5ccの混合液を，0°C〜70°Cの各温度で20分間反応せしめ，前報[2] で述べた如き Nessler 比色法の改良法によつて光電比色し，吸光度に100を乗じて酵素活性として表はした。

b） 実験結果

以上のやうな方法により，K-10 株菌の Urease 活性に及ぼす温度の影響を観察した結果は Fig 2 に示す通りである。すなわち反応温度の上昇につれて活性は増大し 60°C前

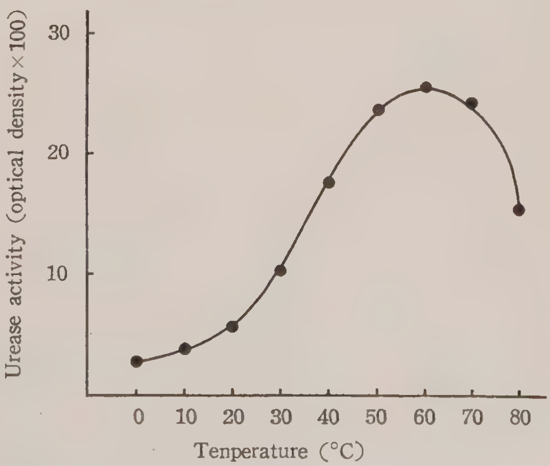


Fig. 2 Effect of temperature on the urease activity of intact cell suspension of K-10 strain

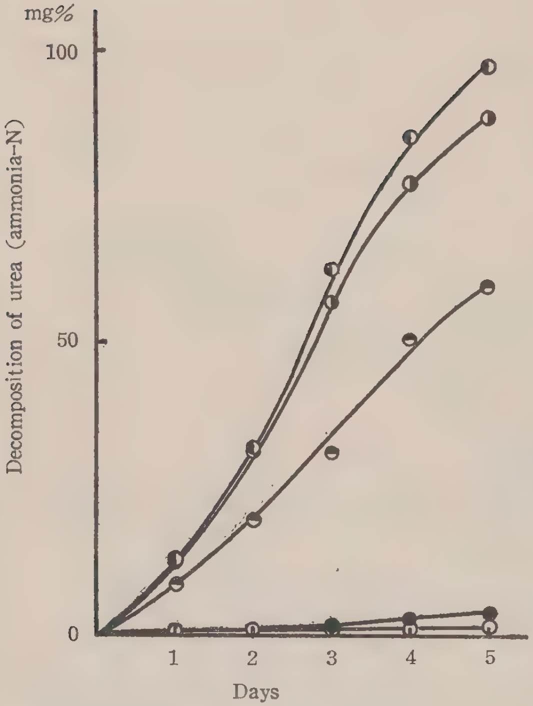


Fig. 3 Urea decomposition at low temperature as −5°C by the cultivated living cell of K-10 strain as follows, ○ not cultivation ● 1 day's cultivation ◐ 2 days' cultivation ◑ 3 days' cultivation ◐ 4 days' cultivation

後において最大の活性を示すが，更に温度が上昇すると急激な活性の減少が見られる。一方低温側においては，30°C前後を境として温度の低下にともなふ活性度の減少は比較的少なく，最低 0°Cにおいても比較的高い活性を示した。

III K-10株菌の −5°C の低温度下における尿素分解能力

a) 実験方法

20cc のペプトン水に一定量の K-10 株菌を接種し 0〜4日間 37°C で培養後，各培養液に終末 2 % となるよう濾過滅菌尿素液を添加し，この混合液を約 − 5°C の冷所に保存し，以後一定時間隔に Ammonia - N の変化を測定して尿素分解の度合を観察した。なお Ammonia-N の測定は Conway の微量拡散分析法[3] によつた。

b) 実験結果

以上のような方法によつて，低温度下における尿素分解能力を観察した結果はFig 3 に示す通りである。すなわち培養日数に比例して，換言すれば菌の発育度に比例して，順次多量の Ammonia-N の発生を示し，4 日培養のものにおいては −5°C の冷所においても 5 日間において100mg%程度の Ammonia-N の発生が観察された。なお尿素無添加のものにおいてはほとんど Ammonia - Nの発生が認められなかつたので，この Ammonia - N の発生はすべて尿素の分解によるものと考えられる。

考　　察

以上の実験結果から次のようなことが考察される。すなわち Fig 1，Fig 2 の結果から本菌の尿素分解能力は40°C 前後において最大を示し，40°C においては発育が非常に悪くなるためその分解能力は急激に減少するものと考えられる。しかし一方 Fig 2 の結果に示されるようにこの菌の Urease 活性は 0 °C の低温においてもなお比較的強いことが認められる。この事実は興味あることで冷蔵保存中の鮫肉が多量の Ammonia - N を生成することと深い関連性があるものと推察される。すなわち或る程度鮮度が低下し，此の種の細菌の発育増殖した鮫肉においては，その後の冷蔵保存によつても尿素の分解は比較的速やかに進行することが想像される。以上のような仮定は Fig 3 の結果により更によく説明される。すなわち予め 4 日間の前培養を行つて，この菌の発育増殖が相当進んだものにおいては，−5°C の低温においても尿素の分解は阻止し得ず冷蔵保存 5 日日において，約 100mg% の Ammonia - N の発生が認められた。これに反し殆ど菌が発育していない若い培養のものにおいては Ammonia - N の発生は極めて僅少であつた。この事実から考えて前記の仮定は妥当であり，前報[1] において著者が推論したように K - 10 株菌の如き尿素分解菌が鮫肉の尿素分解の主要な役割をなしているとすれば，或る程度鮮度が低下してこの種の細菌がすでに或る程度発育増殖してしまつたものにおいては冷蔵保存により鮫肉の尿素分解を阻止することは困難で，逆に云うならば鮮度の良好なものほど尿素分解阻止の効果を上げ得るものと考えられるが，このことについては更に次の機会に実際

に鮫肉を用いて実験を行いより明確な結論を得たいと考えている。なほ Sumner[4] の云う如く Urease は他の植物酵素と同じように至適温度が非常に高く短時間では65°C 附近にあるが，Fig 2 に見られる如く本菌の Urease 活性の至適温度もまた比較的高く60°C前後にあるものと考えられる。

要　　約

腐敗過程における鮫肉より分離した尿素分解菌 K-10 株の尿素分解能力に及ぼす温度の影響について観察し次の如き結果が得られた。

1）K-10株菌の発育増殖は 35°C 前後で最も旺盛で 45°C 或は25°C 以下においてはきわめて緩慢である。

2）本菌の Urease 活性は，60°C 附近で最大を示し，これ以上の高温では急激に不活性化するが 0 °C附近の低温度においては比較的高い活性を示す。

3）1），2），から本菌の尿素分解能力は約 40°C 前後で最大を示すものと考えられる。

4）前培養によりすでに相当程度に本菌が発育増殖したものでは，−5°C の低温度においても尿素分解は比較的急速に進行する。

5）4)から考えて鮫肉の冷蔵効果は，原料の鮮度に左右されるものと思はれる。

終りに臨み，本報告の御校閲及び御指導を賜つた本学谷川教授並に坂井助教授に深甚の謝意を表する。

本論文の要旨は昭和31年度日本水産学会秋季大会（於，広島大学）において報告した。

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紅藻の色素蛋白質に関する研究

第1報　フィコエリスリン，フィコシアニンの精製分離と吸収スペクトル

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Studies on Chromoproteins of Red Algae

1. Preparation and light absorption of phycoerythrin and phycocyanin

Hisanao IGARASHI and Yūzi HOSOYA

Abstract

After the removal of impurity from pigment extraction by alumina adsorption, Phycoerythrin and Phycocyanin were separated from several red algae by means of fractional precipition with ammonium sulfate and by the difference of solubility in water.

The light absorption in the visible spectrum of them was measured; some difference between them was observable.

緒　　言

紅藻の水溶性色素蛋白質として，赤色のフィコエリスリン，青色のフィコシアニンの二種類が，種々の人々によつて分離せられ，そのスペクトルによる特性[1,2]，元素分析[3]，窒素の分布[3]，分子量[4]及び色素部[5,6]，蛋白部[7]の性質について研究されているが，色素部分については未だ明らかにされていない点もあり，蛋白部と色素部の結合状態については全く解明されていない。更に之等の色素の生理的意義についても[8]，推定の域を脱していない状態にもある。著者等は之等の不明の点を明らかにする為に，逐次研究を継続するつもりであるが，その第一段階として，種々の試料を用いて之等色素蛋白質の精製分離を行い，それらの間に多少の知見を得たので，ここに報告する。

実験結果並に考察

用いた試料としては，紅藻類のうち粘質物の比較的少いと思われる，オホソゾ (*Laurencia glandulifera* KÜTZ), アカバ (*Neodilsea yendoana* TOKIDA), ダルス (*Rhodymenia palmata* (L.) GREV.), キブリイトグサ (*Polysiphonia japonica* HARVEY) 及びスサビノリ (*Poprhyra yezoensis* UEDA) 等である。試料を採集後細断し，暗所低温（5°C）にて5日乃至7日間，水で抽出した。それらの粗色素液は5.8乃至6.0の pH を示したが，アカバだけは4.55という値を示し，色素の抽出状態は他のものに比して非常に劣つている。色素の単離精製法としては，古くからは Kylin 氏[1]の硫安による分別沈澱法があるが，最近藤原氏によつて[7] rivanol (2-ethoxy-6,9-diaminoacridinum lactate) を用いて精製分離する方法が行われている。著者等は Kylin 氏法によつたのであるが，その一例として，アカバの場合を Fig. 1に示しておく。（他の試料の場合も同様の操作を行つた。）この場合，Kylin 氏法では，水抽出によつて得た粗色素液を直接硫安による塩析を行つているのであるが，粗色素液を遠心分離後，アルミナを充塡した柱を通過させて不純物を吸着させて除去すると，爾後の塩析の繰返し回数を減少させることが出来る。但しこの際蛋白質の変性ということが考えられるが，Haxo[9]等の報告によると些程影響がない様に思われる。

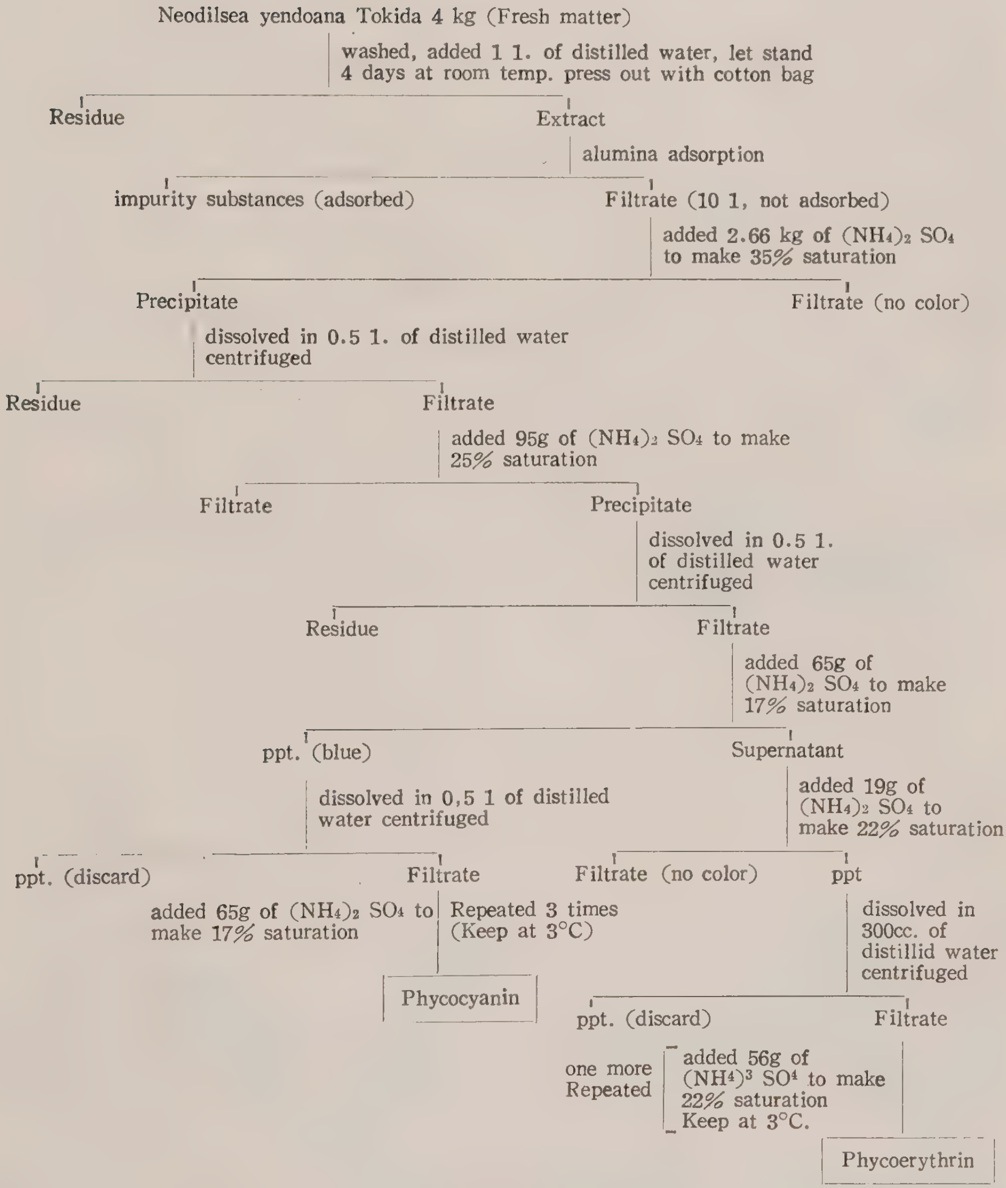


Fig. 1. Scheme for the preparation of Phycoerythrin and Phycocyanin from *Neodilsea yendoana* Tokida

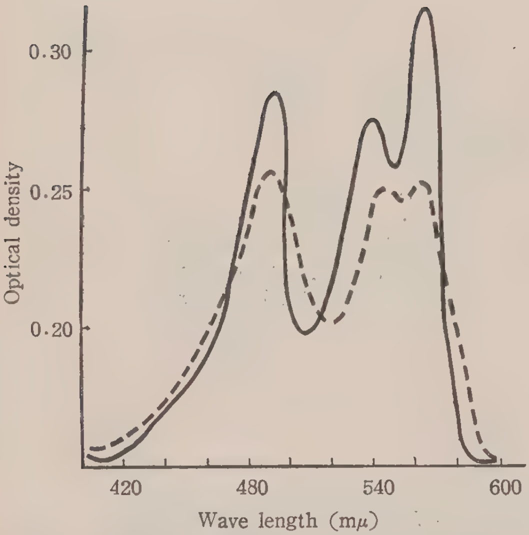


Fig. 2. Spectral absorption curves of Phycoerythrin from *Laurencia glandulifera* Kütz and *Polysiphonia japonica* Harvey
——— *Polysiphonia japonica* Harvey
– – – *Laurencia glandulifera* Kütz

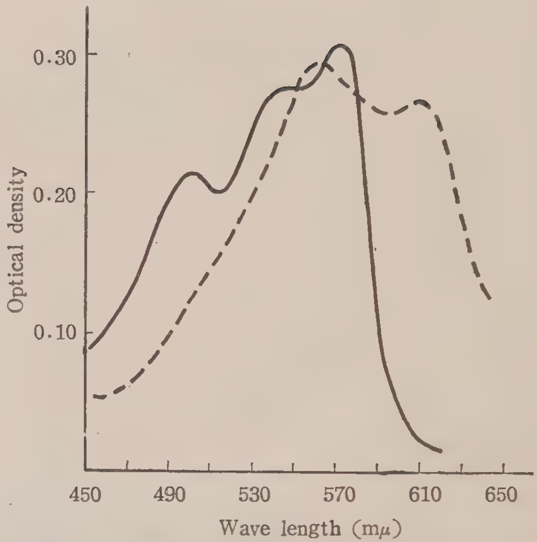


Fig. 4. Spectral absorption curves of Phycoerythrin and Phycocyanin from *Rhodymenia palmata* (L.) Grev.
——— Phycoerythrin – – – Phycocyanin

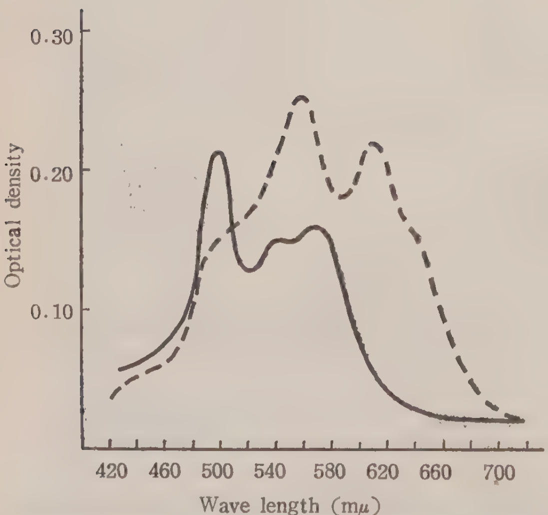


Fig. 3. Spectral absorption curves of Phycoerythrin and Phycocyanin from *Neodilsea yendoana* Tokida
——— Phycoerythrin – – – Phycocyanin

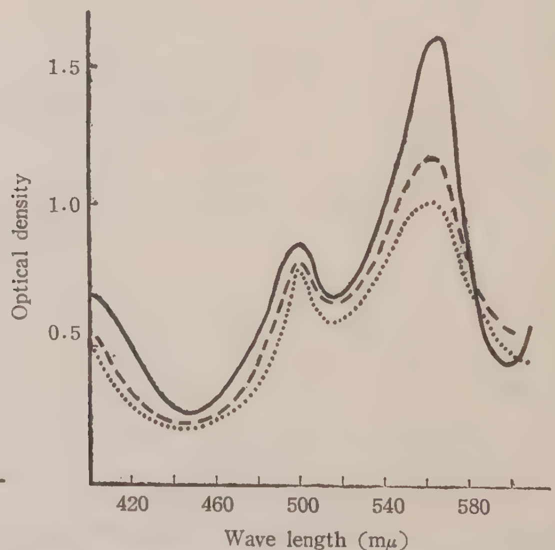


Fig. 5. Spectral absorption of *Porphyra yezoensis* Ueda extracted at various pH values. The indicated pH is that of the final extract.
——— pH8.6 – – – pH6.3 ······ pH4.3

用いた試料のうち，オホソゾとキブリイトグサには，フィコシアニンの存在は認められず，フィコエリスリンのみ得られたが，この様に単独に存在しているフィコエリスリンは，フィコシアニンと共存している場合のそれよりも結晶化しやすい。

上述の様にして得られた色素蛋白質をセロファン紙を用いて5°C に於いて3日間透析を行い，Buffer にて pH を調整し，Beckman spectrophotometer で可視部の吸収スペクトルを測定したが，その結果は Fig. 2，3，4，に示してある。

スサビノリから分離したフィコエリスリンの吸収スペクトルは，一般の R-Phycoerythrin のそれと異り，第2番目の極大吸収が認められなかつた。之は Haxo[9] 等が，*Porphyra perforata J. Ag.* から得たものと類似しているが，Haxo が云うように，変性というよりも，同じ海藻でも成育条件によつて異つた色素蛋白質を形成するということによるのかも知れない。

著者等のあつかつた各試料の吸収スペクトルの極大値を Table 1 に示したが，試料によつて差異のあることが分る。之は従来の研究者の得た結果をも含めて云えることであるが，その原因としては，精製過程に於ける二次的変化によるものか，或は試料それ自体の差異によるものか，今にわかに断定出来ないことのように思われる。

Table 1. Spectral absorption maxima of various red algae

	Algae	Spectral absorption maxima			pH
phycoerythrin	*Neodilsea yendana* Tokida	490	540	570	6–7
	Rhodymenia palmata (L.) Grev.	500	540	570	6–7
	Laurencia glandulifera Kütz	495	545	565	6–7
	Polysiphonia japonica Harvey	496	538	566	6–7
	Porphyra yezoensis Ueda	500		565	6–7
	Porphyra tenera Kjellman[7]	496	546	560	6.8
	Porphyra perforata J. Ag.[9]	492		562	6–7
phycocyanin	*Neodilsea yendana* Tokida	560	610		6–7
	Rhodymenia plamata (L.) Grev.	564	615		6–7
	Porphyra tenera Kjellman[7]		615		6.8
	Porphyra perforata J. Ag.[9]	555	617		6–7

また，抽出条件（pH の変化）による吸収曲線の変化をスサビノリによつて調べた結果を Fig. 5 に示してあるが，之によると pH 4.3—8.6 の間に於いては，その極大値の位置には変化はないが，二つの極大値の吸光係数の比が異つてくることが示されている。Haxo 等の行つた結果では[9]，pH 7.3以上では 562.5mμ に新極大値と，580—585mμ に一つの shoulder が現われることを認めているが，我々に於いては之等の現象は観察されなかつた。

要　約

数種の紅藻より，硫安による分別沈澱法と水に対する溶解度の差を利用して，フィコエリスリンとフィコシアニンを単離精製した。かくて得た両色素についての吸収スペクトルを測定し，その間に差異の存在することを認めた。

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CHEMICAL STUDIES ON HERRING MEAT

Shigeo SASA†

The late Shigeo Sasa, a former Head and Professor Emeritus of the Hakodate College of Fisheries which has eventually become the Faculty of Fisheries of Hokkaido University, passed away in February 1949 due to old age. He was very busy in the management of the Hakodate College of Fisheries and never found opportunity to publish his life work, an investigation on the subject "Chemical Studies on Herring Meat".

The manuscript left by him is still considered of value; it is published in the form of this paper after arrangement, revision and the addition of new data by his follows, Dr. Eiichi Tanikawa, Prof. of Faculty of Fisheries (Hakodate), Hokkaido University, and Mr. Minoru Akiba, Instructor of the same Faculty.

Content

Introduction

The author (Sasa) has previously discussed and published[1] "The Food Values of Fish Meat" from the chemical, physiological and economic points of view. From the viewpoint of chemistry, general chemical constituents, elements, the distribution of nitrogen, fat, and calories of fish meat were discussed; from the physiological point of view, digestibility and absorption of fish meat in experimental animals were discussed; from the economic point of view, the amounts of the catch, and price of fish were discussed from various data in comparison with the meat of domestic animals. As a consequence, it was clarified that the food value of fish meat is as good as that of the domestic animals.

Here, the food value of herring meat, which is one of the abundant catches in Japan, is discussed on the basis of the experimental data on the general chemical

constituents, amount of protein, digestibility by laboratory animals, and on the other data which have been obtained thereafter by other investigators.

I. OUTLINE OF HERRING FISHERY IN JAPAN

Herring is one of the important fishes in Japan. It is caught in Hokkaido, Saghalien, Korea. The distribution of herring was wide in Japanese waters in old times. But recently the amount of the herring catch has been decreasing in that area.

The herrings caught in Japanese waters are considered to be not all of the same family. The family lines are classified into "Japan Sea Family", "Okhotsk Sea Family", "Eastern Saghalien Family", "Pacific coast Family" and "Lakes Family".

In spring, from the end of March to early June, herrings come to the western coast of Hokkaido, viz., from Shiribeshi coast near Yoichi, Hokkaido, to Saghalien waters; to the near-by the islands of Rishiri and Rebun and to the islands Teuri and Yagishiri; to Aniwa Bay (Saghalien) and the near island, Kaiba (Saghalien), and to the whole western coast of Saghalien (See Fig. 1).

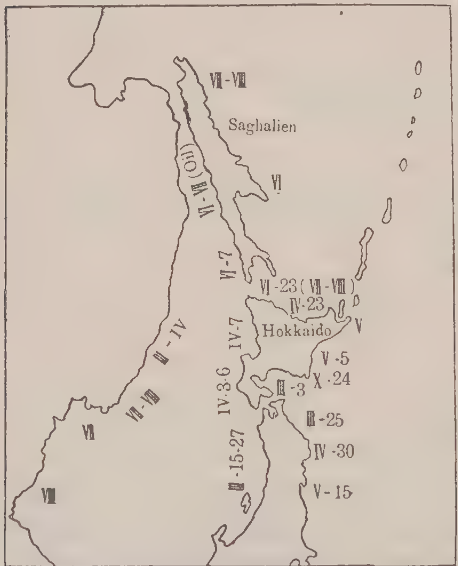


Fig. 1 Map showing the date of initial catching of herring in water of Japan Sea

Those herrings are variously called "Spring herrings" ("Haru-Nishin" in Japanese), "Fore-coming herrings" ("Hashiri-Nishin"), "Intermediate-coming herrings" ("Naka-Nishin"), "Spawning herrings" ("Sanran-Nishin"), "Crowd-coming herrings" ("Gunrai-Nishin"), "Black nose herrings" ("Hanaguro-Nishin"), "White nose herrings" ("Hanashiro-Nishin"), or "Ebisu herrings".

Among those herrings, the main shoal has large bodies, 20～40 *cm* in size, and are corpulent, rich in fat, and has matured roe and milt. They are coming to the coasts for spawning.

In summer, herrings come to the eastern coasts of Hokkaido, especially from Akkeshi to Kushiro and to both eastern and western coasts of Saghalien from early June to August or autumn. They are variously called "Foolish sardine" ("Baka-Iwashi"), "Small herrings" ("Ko-Nishin"), or "Oily herrings" ("Abura-Nishin"). Among those herrings, "Small herrings" are small, 13～20 *cm* in size, poor in fat, whilst "Oily herrings" are large and corpulent and rich in fat. Those herrings are coming to eat food, and are young being 2 or 3 years old. The milt or roe are not yet matured.

In winter, herrings (called "Winter herrings") come to several coasts of Hokkaido on the Pacific Ocean side. The bodies are of various sizes, large or small. Those herrings are said to be remains of summer herrings, therefore they are thin, poor in fat. "Winter herrings" which come to Korean northern coast line, from Seishin to Urusan, are comparatively large, but in those waters the amount is found to decrease as one goes to the south.

Among those three kinds of herrings, "Spring herrings" and "Summer herrings" are the objects for the economic fishery in nowadays. Recently from the decreasing amount of "Spring herrings", the exploitation of fishing grounds of "Oily herrings" in northern Okhotsk has been done[2]; the parental relation between the "Spring herrings" which are caught in Japan Sea and "Small herrings" ("Summer herrings") which are caught along eastern coasts of Hokkaido on the Pacific Ocean side, and the migration of "Small herrings" are under study[3]. According to the results[4] obtained, the "Small herrings" are considered to have no parental relation with "Spring herrings" which come to Japan Sea coasts.

In old times, "Small herrings" which are caught off Kushiro, in Akkeshi Bay ("Lake herring"), near Kayabe, or Lake Obuchi in Aomori Prefecture were considered to be independent families; now, however, it is considered doubtful whether that is fine.[3]

Discussing the quality of the meat, "Autumn herrings" of Saghalien are suitable for salted goods. "Spring herrings" caught off western coast of Hokkaido are spawning herrings, so the roe and milt are over-matured, the amount of fat is small, and the body loses flesh, therefore they are not suitable for salting. However, the "Spring herrings", which are caught 4 or 5 weeks before the spawning are suitable for salting. Those herrings are called especilly "Fore-coming herring" or "Snow season herring" ("Yuki-Nishin").

"Spring herring" is divided into three categories according to the catching season; the first group is from March 21st to April 15th ("Fore-coming herring" or "Snow season herring"), the second group is from April 16th to April 30th ("Intermediate-coming herring"), the third group is from May lst to June 31st ("Late-coming herring") ("Ato-Nishin"). Herrings are abundantly caught in a short period, so the catch is made into "Dried herring meat" ("Migaki-Nishin" in Japanese) which is the simplest merchandise, or fish fertilizer ("Gyokasu"). "Dried herring meat" is made from "Fore-coming herring" or from the early catch of "Intermediate-coming herring".

The "Fore-coming herring" is large, fatty and good in taste. The "Intermediate-coming herring" and "Late-coming herring" are spawned herring or young herring, so the body has lost flesh, or is small sized, therefore they are unsuitable as raw material for the dried herring meat. Especially the "Late-coming herring" has inferior quality flesh. At the catching season, temperature is becoming high, so the herring is liable to

decomposition, therefore it is made into fish fertilizer. The "Spring herrings" which has not yet spawned is salted and smoked. The smoked herring made from such a material is of good quality.

The author has endeavoured to study the making of foods from herring, not the making of fish fertilizer.

II. CHEMICAL COMPOSITION OF "SPRING HERRING"

"Spring herrings" come to the whole western coasts of Hokkaido to spawn. The general chemical components of fish generally vary with season, even if the fish is the same species. Clark and Almy have observed the variation of the chemical components of the migrating fishes[5]; and they have stated that the components, especially fat and water-contents of the meat vary by catching season, age of fish, spawning, nutrition, etc.

The present author in 1925 compared the general chemical components of "Spring herrings" caught near Otaru, Hokkaido by their sex and maturity.

1. Experimental method

The weight and length of the bodies, the weight of milt or roe and the weight of the edible part of herrings which were caught at various times were estimated by comparing the age, sex and maturity. Nextly the amounts of water-content, total nitrogen, crude fat and ash of the meat muscle of the back part were estimated. Samples employed were each 10 ~ 20 fishes.

2. Experimental results

The experimental results obtained are shown as Table 1.

As seen in Table 1, the chemical components of the meat of "Spring herring" varied remarkably by the fishing period. In those experiments, the amount of water content was 67 ~ 78 %, crude protein (Total N × 6.25) was 16 ~ 21 %, crude fat was 1.5 ~ 12 %, and ash was 0.9 ~ 1.7 %. Among the variations of the chemical components, those of water content and crude fat were remarkable.

(*1*) *Periodical variation of the weights of milt or roe and edible part*

Viewing the ratios of the weight of milt or roe and edible part of the "Spring herrings" for the total weight of a fish body in Table 1, one will note that the ratios varied remarkably according to fishing period. But the ratios obtained in Table 1 do not suggest remarkable difference by ages of fishes. Therefore the variations of the ratios are drawn up by difference by sex of the fishes as shown in Figs. 2 and 3.

As seen in Fig. 2, the weight ratio of milt or roe of the "Spring herrings" became remarkably larger at the end of March, and decreased remarkably from early April to the end of April; afterwards the "Spring herrings" have disappeared from May to October, so the experiments could not be carried out. From November to February of

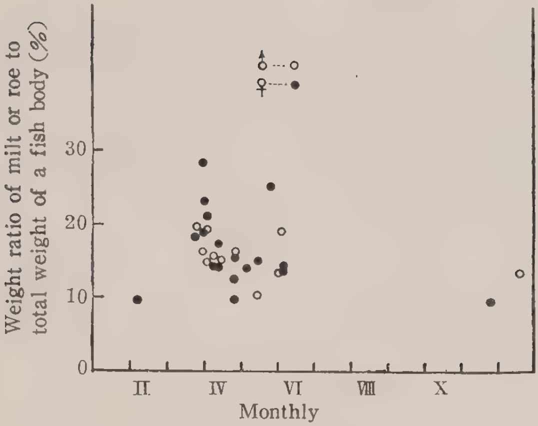


Fig. 2 Variation of the ratios of the weights of milt or roe to the total weight of a fish body by sex

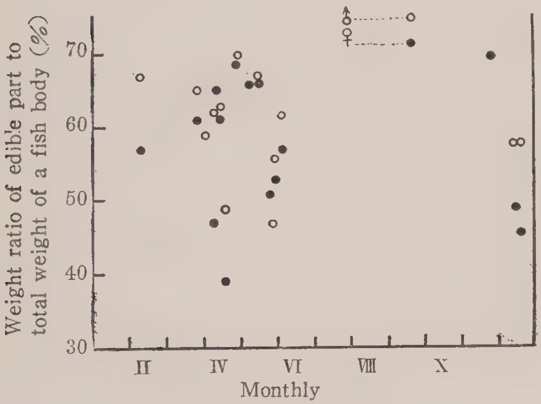


Fig. 3 Variation of the ratios of the weight of the edible part to the total weight of a fish body by sex

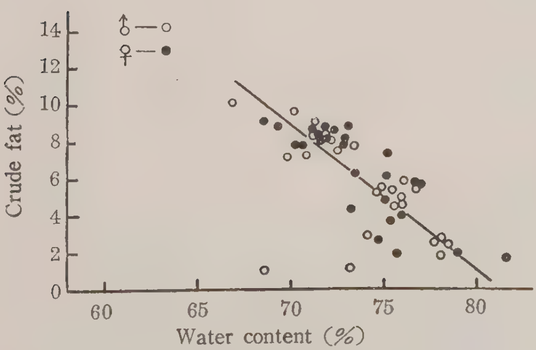


Fig. 4 Relation between the amounts of water content and fat

the next year, the "Spring herrings" were caught as unmatured fishes, so the weight ratio of milt or roe was small. At the end of March, the ratio remarkably increased. As may be noted in Fig. 1 the spawning season of the "Spring herrings" is supposed to be from the end of March to the end of April. That is to say, the weight ratio of milt or roe increased remarkably before the spawning with development toward maturity, and the ratio decreased at the end of April after the spawning.

On the contrary, as seen in Fig. 3, the ratio of the weight of the edible part of the herring showed the maximum value at the end of April. This is due to the exhaustion of milt or roe at the spawning, therefore the ratio of the weight of the edible part increased relatively.

(2) *Periodical variation of the relation between the amounts of water content and fat in the "Spring herrings"*

From Table 1, the fact that when the herrings have larger amount of water content, the amount of fat is smaller, was suggested. The relation between the amounts of water content and fat was graphed in Fig. 4 by the sexual difference of the "Spring herrings" which were caught at various times.

As seen in Fig. 4, there was no remarkable difference by sex be-

tween the relations of the amounts of water content and fat. The relations were shown as a straight line. That is to say, when the amount of water content increased in the meat of the herrings, the amount of fat decreased. This fact has been afterwards clarified by many investigators, *e.g.* in the meat of mackerel by Miyama and Saruya[6].

The periodical variations of the amounts of fat and water content differing from the sex of fishes are shown as Figs. 5 (1), (2) and Figs. 6 (1), (2).

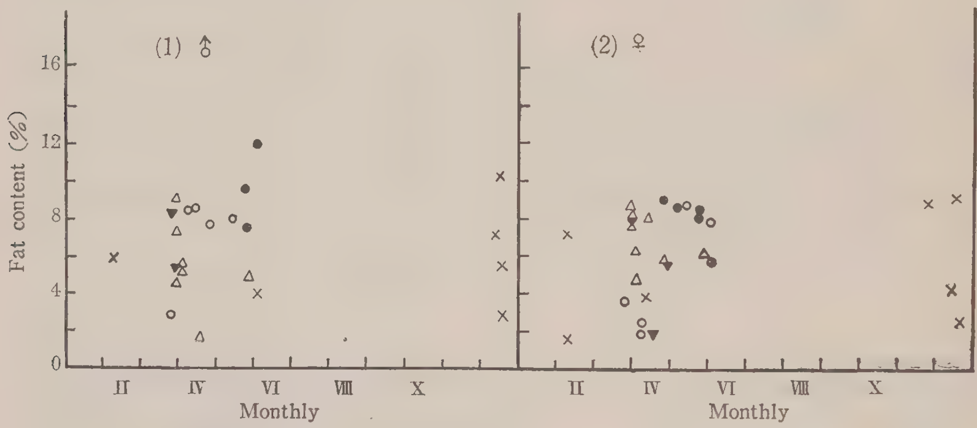


Fig. 5 Periodical variation of fat content

(1)……male, (2)……female
×…3 years old, ●…4 years old, ○…5 years old, △…6 years old, ▼…7 years old

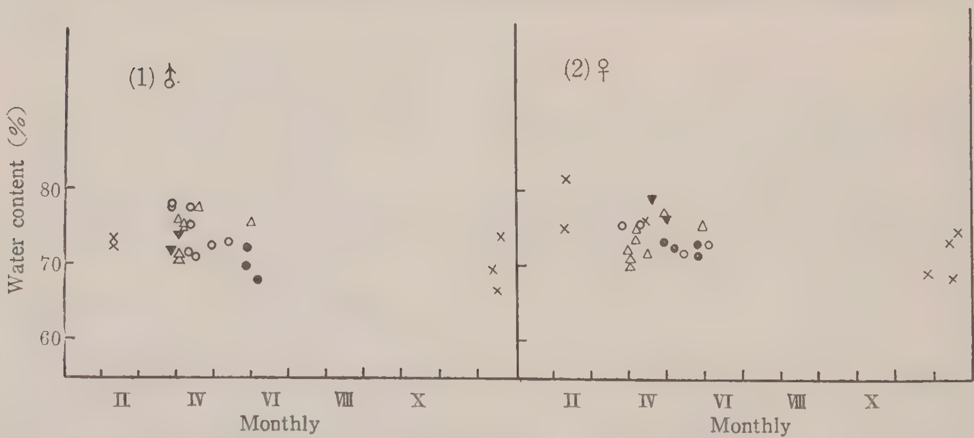


Fig. 6 Periodical variation of water content

(1)……male, (2)……female
×…3 years old, ●…4 years old, ○…5 years old, △…6 years old, ▼…7 years old

In Figs. 5 and 6, the dates, at which the herrings employed for sample by the difference of ages were caught, were irregular, to the author's regret, so the data have not been discussed in detail. But generally speaking of plots in Figs. 5 and 6, taking into account the contrary relation between the amounts of water content and fat contents in the

meat of the herrings, the amount of the crude fat attained larger value at the end of March, in the fishes of 3, 5, 6 and 7 years old, and decreased in the middle of May. The fishes of 4 years old showed the larger amount of crude fat at middle of May.

Considering from the results, as above mentioned, the amount of crude fat increased before the spawning, and decreased after the spawning, and then increased again with regaining of the body strength. The variations in the amounts of water content and crude fat from November to February of the next year were not clear, because the sample fish were obliged to limit to 3-year-olds and the number was small.

(3) *Periodical variation of the amounts of crude protein and ash in the meat*

From Table 1, the periodical variations of the amounts of crude protein and ash in the meat by the difference of sex are shown as Figs. 7 (1), (2) and Figs. 8 (1), (2).

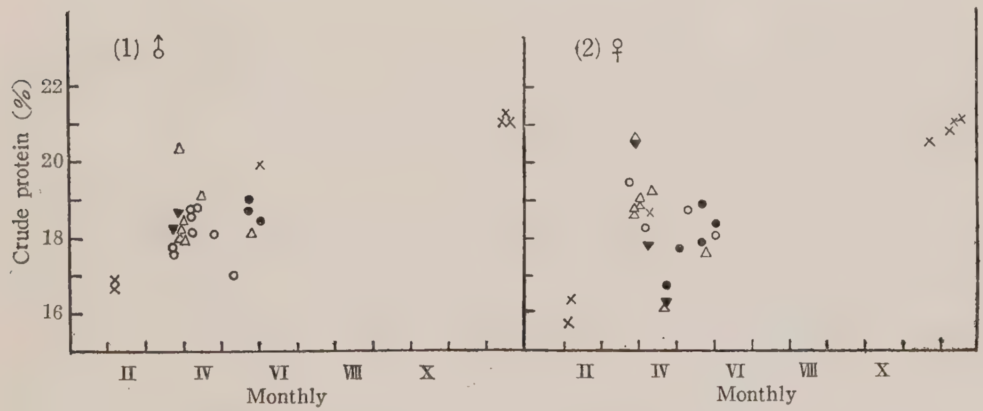


Fig. 7 Periodical variation of the amount of crude protein

(1)……male, (2)……female

×…3 years old, ●…4 years old, ○…5 years old, △…6 years old, ▼…7 years old

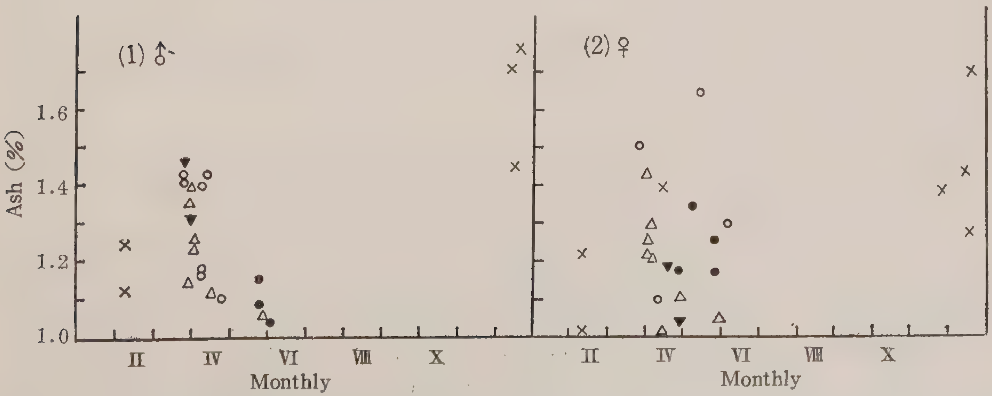


Fig. 8 Periodical variation of the amount of ash

(1)……male, (2)……female

×…3 years old, ●…4 years old, ○…5 years old, △…6 years old, ▼…7 years old

As seen in Figs. 7 and 8, the variation of the amounts of crude protein and ash in the meat of the male and female herrings was in accordance with the periodical variation of the amount of crude fat. That is to say, in fishes of 3, 5, 6 and 7 years old, the amounts of crude protein and ash showed the largest amount at the end of March, then decreased to the middle and end of April, and increased gradually in May. In fishes of 4 years old, the amounts of crude protein and ash increased from the end of April to the end of May, though the amounts in March were unknown, because no samples were obtained. But from the fact that the amounts showed comparatively larger values from November to December, it is suggested that the amounts increased from June to the winter season. The amounts are suggested to decrease again in winter to February, and to increase until March before the spawning.

3. Considerations

From the periodical variations of general chemical components of the meat of "Spring herrings", the amounts of water content and crude fat may be said to vary remarkably. Those components showed always the contrary relation; the sum of those components showed almost the same value (80 ± 3 %) through the year.

The amounts of crude fat, crude protein and ash in the meat decreased remarkably from March to April which are the spawning months judging from the variation of the weight of milt or roe. From the fact that before and after the period, the amounts show the maximum, it is judged that the components accumulate in the meat before the spawning, and decrease at the spawning, and then increase again with regaining of physical strength of the body. Miyama and Saruya[6] have observed that in mackerel meat, the amount of crude fat decreased gradually from April to May which are the spawning season, showed the minimum value from May to July, and afterwards increased reaching the maximum value at January to February of the next year. Fukuhara[7] has compared the amounts of chemical components of salmon meat before and after the spawning. He observed that the amounts of crude fat and lecithine decreased after the spawning, and the amounts of arginine and histidine in amino acids decreased, but the amount of lysine increased.

The present author has also observed the decrease of histidine and the increase of lysine after the spawning as in the results presented below (Ref. Article VIII).

According to Dill's result[8] obtaining from the estimation of the general chemical composition for the "Small herrings" caught monthly, the amounts of crude fat showed the maximum value in the period from July to September. Lovern and Wood[9] have studied the periodical variation of the chemical components of herrings in the Scotia Sea and the Shetland Islands. In those herrings the amount of the crude fat showed the minimum value from March to April, that is to say, the amount was about 5 % in the unmatured herrings (3 years old) and adult (7 years old), but the amount increased

remarkably from July to August, and attained the maximum value, that is to say, about 20 %. But the amount of the fat content decreased to April of the next year. Those variations are suggested to be due to the spawning (in August). Before the spawning the amount of crude fat was larger and at the spawning, the amount decreased by the fasts. They have also observed that the amount of the crude fat depends upon the kind of plankton eaten. At the period of the increasing of the amount of the crude fat, the herrings have abundantly eaten plankton having rich fat, *Calanus finmarchicus*.

From the results obtained, the periodical variation of the amounts of crude fat, protein depends clearly on the spawning of the herrings also in Japan.

III. CHEMICAL COMPOSITION OF THE "SMALL HERRINGS"

The name "Small herrings" is applied to those herrings which are caught from Akkeshi to Kushiro of Hokkaido and both eastern and western coasts of Saghalien in summer, as above noted.

The author has determined the amount of edible part, general chemical composition and the distribution of the nitrogens of the so-called "Small herrings" which are caught off Kushiro.

(1) *The amount of edible part of the "Small herrings"*

Each 3 fishes of male and female of the "Small herrings" were used and the amount of edible part was determined. The resutls obtained are shown as Table 2.

Tabe 2. Weight ratio of edible part of the "Small herrings"

Sex	Items	Sample 1	Sample 2	Sample 3
♂	Body weight (*A*)	37.9 *g*	63.0	19.1
	Weight of edible part (*B*)	24.4 *g*	40.5	12.8
	Weight ratio of edible part ($^B/_A \times 100$)	64%	64	67
♀	Body weight (*A*)	37.1 *g*	65.3	45.8
	Weight of edible part (*B*)	22.5*g*	39.8	28.1
	Weight ratio of edible part ($^B/_A \times 100$)	61%	61	61
Remarks	Herrings used were caught in the middle of July.			

As seen in Table 2, male fish have larger amount of edible part than female. In general, the weight ratios of the edible part of fishes have been found to be 30 ~ 70%. The weight ratio of the edible part of the "Small herring" is larger than that of other

fishes.

(2) *General chemical composition of "Small herrings"*

Three males and 4 females of "Small herrings" which were caught off Kushiro were used for the determination of the amounts of water-content, total nitrogen, fat and ash by usual methods. Results obtained are shown as Table 3.

Table 3. General chemical composition of the meat of "Small herrings"

Sex	Sample No.	Date of catching	Age	Water (%)	Crude protein (%)	Crude fat (%)	Ash (%)
♂	No. 1	7th, July	2 years old	59.2	20.6	18.9	1.22
	No. 2	ibid	2	75.7	19.8	3.5	1.01
	No. 3	16th, July	2	71.1	20.0	7.9	1.04
♀	No. 1	7th, July	2	73.0	19.5	6.3	1.15
	No. 2	ibid	2	61.3	20.5	17.0	1.20
	No. 3	16th, July	2	73.5	19.8	5.6	1.10
	No. 4	17th, July	3	68.9	19.4	10.6	1.18
Average on 2 years old				69.3	19.9	9.6	1.15

As seen in Table 3, the "Small herrings" have 69 % of water-content, 20 % of crude protein, 10 % of crude fat and 1 % of ash on the average. The female has a larger amount of the water-content than the male, but the former has less amount of fat than the latter. Of course, the chemical composition of the fish meat varies generally with the catching season, as above described in Article II, "Spring herrings".

According to Dill[8], small herrings of Newfoundland have the maximum amount of crude fat and the minimum amount of water content in summer, from June to September. Lovern and Wood[9] have also observed that the amount of fat in herrings of the North Sea is the minimum in April, increased remarkably from May and reached the maximum in July and to the spawning time.

"Small herring" which are caught off Kushiro and Akkeshi in early June to August are not found in other seasons. Furthermore, the parental relation between the "Small herring" of the Pacific coast side and "Spring herring" of the Japan Sea is not yet known[3]. Therefore the seasonal change of the chemical components of "Small herring" can not be known.

(3) *Nitrogen distribution of "Small herrings" meat*

"Small herrings" meat which were the same as the sample used for the determination of general chemical components was employed for the determination of the distribution of nitrogen. The meat was treated with water, alcohol, ether and the meat protein was obtained. The meat protein was hydrolyzed with sulfuric acid; the amounts of total nitrogen and nitrogens of amino acid, basic, ammonia and melanin forms were determined. The results obtained are shown as Table 4.

Table 4. Nitrogen distribution in the "Small herrings" meat protein

Items	In dried matter of sample	In ash-free dried matter	In total-N
Total-N	14.73%	15.98%	100 %
Amino-N	9.20	9.98	62.45
Basic-N	4.59	4.98	31.16
Ammonia-N	0.64	0.69	4.92
Melanin-N	0.30	0.33	2.07

As seen in Table 4, in the total amount of nitrogen of the meat protein of "Small herrings", greatest part is supplied by amino acid nitrogen and basic nitrogen.

Lately, Igarashi[10] has estimated the amounts of various nitrogens in the raw herring meat and stated that the amount of total nitrogen was 15.45 %, ammonia nitrogen was 1.42 % (9.2 % of the amount of the total nitrogen), melanin nitrogen was 0.28 % (1.8 % of the same), monoamino nitrogen was 8.46 %, (54.8 % of the same), diamino nitrogen was 5.28 % (34.2 % of the same) in the ash free anhydrous sample. Sekine[11] has also estimated the same components with 22 kinds of fishes, and found that the amount of total nitrogen was 16.22 %, amino acid nitrogen 10.09 %, basic nitrogen 4.92 % and ammonia nitrogen was 0.92 % on the average. By comparing with those data, one may see that the amounts of the nitrogenous components of the "Small herrings" meat protein are the same as those of other fishes.

IV. CHEMICAL COMPOSITION OF "OILY HERRINGS" CAUGHT AT VARIOUS PLACES

The general chemical compositions of the so called "Oily herrings" were determined by usual methods. Several fishes of male and female caught off Kabuka on Rebun Island, Wakkanai, Kitami-Esashi, Saghalien, Vladivostock and Kamchatka in the U.S.S.R., Korean coasts, Pacific side of the U.S.A. (Berkeley) were used for the determinations of water-content, total nitrogen, crude fat and ash.

1. Experimental results

(1) Herrings caught off Rebun Island

Each 3 fishes of male and female caught off Rebun Island on 6th June were used. Results are shown as Table 5.

(2) Herrings caught near Wakkanai

Each 3 fishes of male and female 6 years old caught near Wakkanai on 21st June were used. Results are shown as Table 6.

(3) Herrings caught off Esashi, Kitami district

Two male and three female of herrings caught off Esashi, Kitami district of Hokkaido were used. Results are shown as Table 7.

Table 5. General chemical components of "Oily herrings" caught off Rebun Island

Sex		♂			♀		
Sample No.		1	2	3	1	2	3
Water (%)		68.1	68.3	68.2	68.4	68.7	68.7
Crude protein (Total-N × 6.25) (%)		18.0	17.9	17.8	17.7	17.6	17.5
Crude fat (%)		12.6	12.5	12.7	12.4	12.3	12.4
Ash (%)		1.27	1.20	1.31	1.48	1.39	1.37
Remarks	Body length	30 *cm*	30	30	32	30	30
	Body height	8 *cm*	7	7	8	8	8
	Body weight	200 *g*	163	159	210	174	242
	Ages	7 years old	6	6	6	6	6

Table 6. Herrings caught near Wakkanai

Sex		♂			♀		
Sample No.		1	2	3	1	2	3
Water (%)		69.1	69.5	70.0	70.8	70.6	70.9
Crude protein (Total-N × 6.25)(%)		18.1	17.9	17.8	17.4	17.6	17.6
Crude fat (%)		11.3	11.2	11.0	10.5	10.3	10.2
Ash (%)		1.33	1.38	1.21	1.39	1.43	1.46
Remarks	Body length Body weight	27 *cm* 196 *g*	26 176	26 185	26 185	26 185	26 180

Table 7. Herrings caught off Esashi, Kitami district

Sex	♀			♂	
Sample No.	1	2	3	1	2
Water (%)	74.2	74.7	74.4	73.1	73.7
Crude protein (Total-N × 6.25) (%)	19.6	19.5	19.5	20.3	20.0
Crude fat (%)	5.1	4.5	5.0	5.2	4.9
Ash (%)	1.10	1.26	1.13	1.33	1.38
Ages	6 years old	5	5	5	5
Remarks	Caught by set net, 1st, May			Caught by gill net, 28th, May	

(4) *Herrings caught off western coast of Saghalien*

Each 3 males and females of herrings caught on 21st July and each 2 males and females caught on 22nd July were used. All these herrings were 7 years old. Results are shown as Table 8.

Table 8. Herrings caught off western coast of Saghalien

Items \ Sex		♂			♀		
Caught in 21st, July	Sample No.	1	2	3	1	2	3
	Water (%)	61.9	61.9	61.7	63.4	63.9	63.4
	Crude protein (%)	17.8	17.7	17.9	16.5	17.4	17.5
	Crude fat (%)	19.1	19.2	19.1	17.8	17.3	17.9
	Ash (%)	1.20	1.26	1.25	1.33	1.35	1.35
	Note: Body length Body height Body weight	31 *cm* 7 *cm* 255 *g*	31 7 244	30 7 263	30 7 263	30 7 251	30 7 251

Items \ Sex		♂		♀	
Caught in 22nd, July	Sample No.	1	2	1	2
	Water (%)	69.0	69.4	69.0	69.2
	Crude protein (%)	17.0	17.4	16.9	17.3
	Crude fat (%)	12.6	11.9	12.8	12.4
	Ash (%)	1.34	1.27	1.39	1.18

(5) *Herrings caught off Vladivostock*

Two males caught in the middle of February and two females caught late in March were used. Results are shown as Table 9.

Table 9. Herrings caught off Vladivostock

Sex	♂		♀	
Sample No.	1	2	1	2
Water (%)	68.4	68.5	67.8	69.2
Crude protein (Total-N × 6.25) (%)	19.2	19.1	19.3	19.0
Crude fat (%)	11.0	11.0	11.0	10.0
Ash (%)	1.40	1.41	1.84	1.75
Remarks	Taken in middle of February, male, 3 years old		Taken in end of March, female, No. 3 (6 years old) No. 4 (5 〃 〃)	

(6) *Herrings caught off western coast of Kamchatka Peninsula*

Each 3 males and females caught in August were used. Results are shown as Table 10.

Table 10. Herrings caught off western coast of Kamchatka

Sex		♂			♀		
Sample No.		1	2	3	1	2	3
Water (%)		67.5	68.1	67.6	70.1	70.3	69.8
Crude protein (Total-N × 6.25) (%)		18.1	18.0	17.9	17.8	17.8	17.6
Crude fat (%)		13.0	13.1	13.1	10.8	10.4	11.1
Ash (%)		1.35	0.83	1.38	1.40	1.48	1.44
Remarks	Body length	26 *cm*	26	26	26	26	26
	Body height	6 *cm*	5	7	5	5	5
	Body weight	109 *g*	109	138	107	109	109

(7) *Herrings caught off Korea*

Three males and 6 females of herrings caught off the northern coast of Korea in the middle of February were used. Results are shown as Table 11.

Table 11. Herrings caught off Korea

Sex	Sample	Water (%)	Crude protein(%)	Crude fat (%)	Ash (%)	Note
♀	No. 1	64.8	17.5	16.5	1.20	3 years old
	No. 2	66.5	18.5	13.6	1.45	〃
	No. 3	66.1	18.6	13.9	1.35	4 years old
	No. 4	65.0	18.3	15.4	1.37	Spent
	No. 5	61.6	18.0	18.0	1.46	〃
	No. 6	60.3	20.8	17.6	1.35	Full matured
♂	No. 1	69.4	17.9	11.3	1.46	3 years old
	No. 2	66.4	18.8	13.4	1.43	Spent
	No. 3	63.2	20.7	14.7	1.42	Full matured

(8) *Herrings caught on the Pacific coast of the U.S.A.*

Four males and 1 female of herrings of 4 years old caught off Berkeley in January were used. Those samples were salted to bring them to the author's laboratory without decomposition. On this herring, the amount of NaCl in the meat was estimated. Results are shown as Table 12.

Table 12. Herrings caught on the Pacific coast of the U. S. A.

Sex	Water (%)	Crude protein (%)	Crude fat (%)	Ash (%)	NaCl (%)
♂	53.5	21.1	16.6	15.8	14.4%
〃	53.9	21.1	16.9	16.0	14.6
〃	53.9	20.8	17.2	15.9	14.5
♀	51.8	20.6	15.9	15.3	14.0

Table 13. Average value of the the general chemical components of "Oily herrings" caught at various places

Catching place \ Items	♂ Water (%)	♂ Crude protein (%)	♂ Crude fat (%)	♂ Ash (%)	♀ Water (%)	♀ Crude protein (%)	♀ Crude fat (%)	♀ Ash (%)	Remarks
Off Rebun Island	68.2	17.9	12.5	1.26	68.6	17.6	12.3	1.41	Caught in the first of June; 6 and 7 years old
Near Wakkanai	69.5	17.9	11.2	1.91	70.7	17.5	10.3	1.43	Caught in the end of June; 6 years old
Off Kitami-Esashi	74.0	19.8	4.9	1.24 ···(Sex was unknown)					Caught on May
Western coast of Saghalien	65.5	17.5	15.7	1.27	66.4	17.1	15.2	1.32	Caught in the end of July; 7 years old
Off Vladivostock	68.5	19.2	11.0	1.41	68.5	19.2	10.5	1.80	♂ (Caught in the middle of Feb.; 3 years old) ♀ (Caught in end of March; 5 and 6 years old)
Western coast of Kamchatka	67.7	18.0	13.1	1.19	70.1	17.7	10.8	1.44	Caught on August; 4 years old
Off Korea	67.9	18.4	12.4	1.44	64.8	18.2	15.5	1.37	Caught in the middle of Feb.; 3 and 4 years old

2. Considerations

From these data obtained with herrings caught at various places, the average value of the general chemical components are shown as Table 13.

From Table 13, in so-called "Oily herrings" meat there is above 10 % of crude fat in the fish meat excepting the herring caught off Esashi.

Comparing with "Small herrings" of which the general chemical components have been described above in Article III, the "Oily herrings" have greater amount of the crude fat. In general, the male fish have larger amount of crude fat than the female, and contrarily the former has smaller amount of water content than the latter.

V. ON THE CHEMICAL COMPOSITIONS OF THE EXTRACTIVE OF HERRINGS MEAT

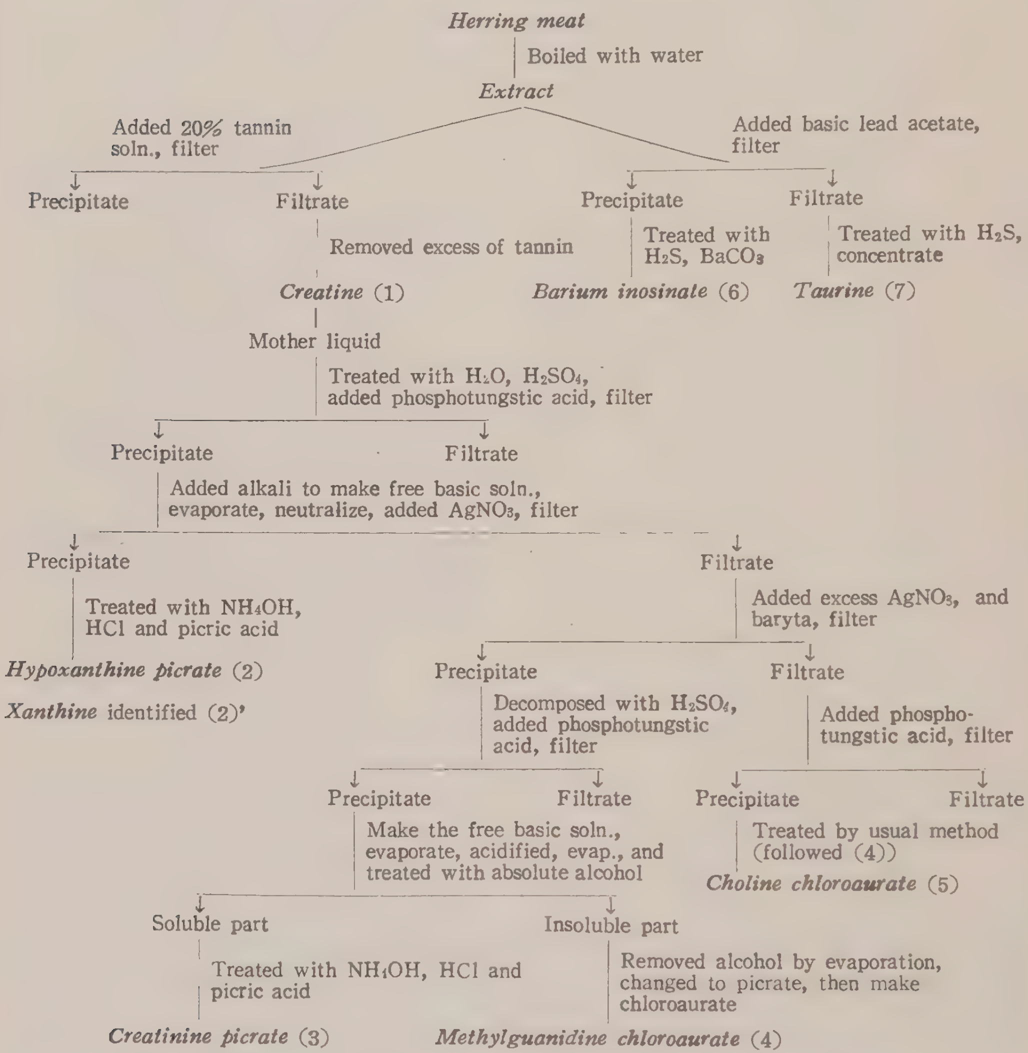
Creatine, creatinine, hypoxanthine, xanthine, methylguanidine, choline, inosinic acid and taurine were isolated from the meat extractive of herrings which were caught off Yoichi, Hokkaido, and their amounts were estimated. (See Scheme 1).

1. Experimental results

(*1*) *Creatine*

To one *kg* of the meat of the "Spring herring" (Adult, water-content 71.2 %) caught at the end of April distilled water was added, and boiled. To the total extractive from the boiled meat was added 20 % tannin solution as a protein precipitant. After the removal

Scheme 1. Isolation of various components from the extract of herring meat



of the excess tannin, 2.0110 *g* of colorless, rhumboid, columnar crystals of creatine was obtained. After the drying of the crystal, it was analyzed. From 0.2014 *g* of the sample, 0.0650 *g* of nitrogen was determined. The calculated amount of nitrogen in creatine ($C_4H_9N_3O_2$) was 32.06 %, but the experimental finding was 32.27 %.

(2) *Hypoxantine and Xanthine*

Mother liquid from which creatine had been removed was treated with water and sulfuric acid, then to it was added phosphotungstic acid. From the precipitate, free basic solution was made by usual method. The solution was concentrated by evapo-

ration. After the neutralization of the concentrate, silver nitrate solution was added. The precipitate yielded was treated with ammonia, hydrochloric acid and picric acid by turns. Here 1.3443 *g* of picrate of hypoxanthine was obtained. At this time, the presence of xanthine was slightly observable. From 0.1908 *g* of the sample of hypoxanthine picrate, 0.0092 *g* of water of crystallization and 0.1150 *g* of picric acid were obtained. Whilst the calculated amounts of water are 4.7 % and 59.79 % for picric acid in hypoxanthine picrate ($C_5H_4N_4O \cdot C_6H_3N_3O_7 + H_2O$), the experimental amounts were 4.82 % of water, 60.27 % of picric acid. Thus 0.501 *g* of hypoxanthine was obtained from 1 *kg* of the raw meat of herring.

(3) *Creatinine*

To the filtrate from the precipitate by silver nitrate was added exess of silver nitrate and baryta. The precipitate was decomposed by sulfuric acid. After the addition of phosphotungstic acid, the precipitate yielded was treated by the usual method to make the concentrated solution of free bases. The concentrate was acidified and furthermore evaporated. The concentrate was treated with absolute alcohol and divided into two parts. From the dissolved part, 0.7712 *g* of columnar crystals of creatinine picrate was obtained, *m.p.* 214° ~ 215°C. Then the amount of nitrogen of 0.0501 *g* was estimated from 0.2031 *g* of the crystal. Compared with 24.54 % calculated amount of nitrogen in creatinine picrate ($C_4H_7N_3O \cdot C_6H_3N_3O_7$), 24.67 % nitrogen was found. Therefore, 0.2548 *g* of creatinine was obtained from 1 *kg* of the raw meat of herring.

(4) *Methylguanidine*

From the undissolved part of the material described in previous section (3), alcohol was removed by evaporation. The residue was changed to picrate, then to the complex salt of hydrochloride-chloroaurate. Thus 1.8295 *g* of methylguanidine chloroaurate was obtained. From 0.1129 *g* of the dried sample, 0.053 *g* of gold and from 0.1042 *g* of the same sample, 0.0095 *g* of nitrogen were estimated. Whilst the calculated amount of Au are 47.74 % and 10.16 % for nitrogen in methylguanidine chloroaurate ($C_2H_7N_3 \cdot HCl \cdot AuCl_3$), the experimental amount were 47.74 % of Au, 9.12 % of nitrogen. Thus 0.3381 *g* of methylguanidine was obtained from 1 *kg* of the raw meat of herring.

(5) *Choline*

To the filtrate obtained by the filtration of the precipitate yielded by the addition of silver nitrate and baryta as described in (3), was added phosphotungstic acid. The precipitate yielded was treated by usual method and 1.0051 *g* of columnar crystal of choline chloroaurate was obtained. From 0.1385 *g* of the sample, 0.617 *g* of Au in choline chloroaurate was estimated. Calc. Au in choline chloroaurate, 44.49 %; found 44.55 % experimentally. Therefore 0.3170 *g* of choline was obtained from 1 *kg* of the raw meat of herring.

(6) *Inosinic acid*

The extractive solution from 1 *kg* of raw fresh meat of herring from which reddish meat was removed was treated with basic lead acetate. The precipitate yielded was treated with sulfuretted hydrogen, and barium carbonate. Thus 0.2746 *g* of barium inosinate was obtained. From 0.2180 *g* of the sample, 0.0829 *g* of $BaSO_4$ was estimated. Calc. $BaSO_4$ in barium inosinate, 22.39 %; found 22.41 % experimentally. Therefore 0.1980 *g* of inosinic acid was obtained from 1 *kg* of the fresh meat.

(7) *Taurine*

The filtrate of the precipitate by basic lead acetate as described in (6) was treated with sulfuretted hydrogen and then concentrated. Thus 0.9025 *g* of taurine was obtained. From 0.1403 *g* of the sample, 0.0162 *g* of nitrogen was estimated. Compared with 11.55 % calculated amount of nitrogen in taurine ($C_2H_7NSO_3$), 11.20 % of nitrogen was found.

2. Considerations

The isolation of the chemical components as above described from the extract of the meat of herrings was repeated three times. The experimental results obtained are shown as Table 14.

Table 14. Chemical composition of the extract of the meat of herrings (*g*/*kg* fresh meat)

Components	1	2	3	Average
Creatine	2.0110 *g*	2.0974	1.9749	2.0278 *g*
Hypoxanthine	0.5010	0.5323	0.4864	0.5066
Creatinine	0.2548	0.2521	0.2627	0.2565
Methylguanidine	0.3381	0.3229	0.3335	0.3315
Choline	0.3170	0.3158	0.3184	0.3171
Inosinic acid	0.1980	—	—	0.1980
Taurine	0.9025	—	—	0.9025
Xanthine	+	+	+	+

Previously Yoshimura *et al.*[12] have isolated hypoxanthine, creatine, methylguanidine, choline, cadaverine, putrescine and ammonia from herring meal. Therefore, also in the extract of the raw meat of herring, those components are suggested to occur. The present author has isolated and estimated the components which are shown in Table 14.

The chemical components in the extract of herring meat which were estimated by the present author are compared with the components which have been determined by many investigators in the extract of the meat of other kinds of fish, as shown in Table 15.

As seen in Table 15, the amounts of creatine and creatinine contained in the extract of herring meat are less than those in other kinds of fish. But the amounts of hypoxanthine and methylguanidine contained in the former are considered to be larger

Table 15. Chemical components in the extract of the meat of various kinds of fish (g/kg fresh meat)

Components \ Fishes used	Herring	Bonito	Mackerel	Sardine	Tuna	Salmon
Creatine	2.03	6.49[13]	4.87[13]		4.97[13]	5.60[13]
Hypoxanthine	0.51	0.08[14]		0.50[15]		0.28[16]
Creatinine	0.26	1.34[13]	0.04[13]	0.31[17]	0.64[13]	0.67[13]
Methylguanidine	0.33	0.05[14]				
Choline	0.32					
Inosinic acid	0.20	0.42[14]				
Taurine	0.90	0.80[18]				
Xanthine	+					

Note: Number in parenthesis shows the No. of literature cited.

than that in the latter. Some investigators have estimated 1.6 g of carnosin[19], 0.07 ~ 0.12 g of inositol[20], 0.192 g of trimethylamine oxide[21] in one kg of raw fish meat.

Okuda[22] has isolated purine bases and other components from the extract of the ordinary meat and reddish meat ("Chiai"-meat) of bonito. The present author has also isolated purine bases from the crude meat protein prepared from herring meat. At first, the herring meat ("Fore-coming herring") was treated with water, alcohol and ether. Then 1 kg of the crude meat protein thus prepared was hydrolyzed with sulfuric acid. The hydrolyzed solution was neutralized with baryta, and filtered. The filtrate was concentrated by evaporation. Thus purine bases were obtained as described previously. Then methylguanidine was isolated. From the solution from which methylguanidine was removed, xanthine and hypoxanthine were obtained.

The estimated values are as follows; (1) Methylguanidine was 0.7572 g from 1 kg of herring meat protein as the ash free anhydrous matter. In the sample obtained as methylguanidine chloroaurate, from 0.1528 g of the sample, 0.0728 g of Au (47.64 % of Au) and from 0.1642 g of the sample, 0.0168 g of N (10.23 %) were obtained. The calculated amount of Au is 47.74 % and that of N is 10.23 % in methylguanidine chloroaurate ($C_2H_7N_3 \cdot HCl \cdot AuCl_3$). (2) Hypoxanthine was 0.8439 g from 1 kg of the crude meat protein as the ash free anhydrous sample. In the sample obtained as hypoxanthine picrate, from 0.3071 g of it, 0.0146 g of H_2O (dried at 120°C) (4.82 % of H_2O) and from 0.3031 g of it, 0.1842 g of picric acid (60.78 % of picric acid) were obtained. The calculated amount of H_2O is 4.70 % and that of picric acid is 59.79 % in hypoxanthine picrate ($C_5H_4N_4O \cdot C_6H_3N_3O_7 + H_2O$). (3) Xanthine was slightly observable.

VI. INORGANIC COMPONENTS IN HERRING MEAT AND BLOOD

1. Inorganic components in the meat

(1) General inorganic components in herring meat

Fresh meat of "Fore-coming herring" was chemically analyzed, and the obtained

results are as shown in the 1st column of Table 16.

Table 16. Inorganic components in herring meat and comparison with that of other fish meats (% in the dried matter of the meat)

Components \ Fishes	Herring	Tuna	Sardine	Mackerel	Bonito[22]	Cod[27]
S	0.061	1.40[23] 1.25[24]	0.97[23] 1.06[24]	1.08[24]	—	—
Ca	0.095	0.14[23]	1.48[23]	—	0.121	0.114
Fe	0.015	0.04[25]	0.04~0.05[25]	—	0.013	0.030
Mg	0.129	0.87[23]	0.70[23]	—	0.109	0.086
Na	0.299	—	—	—	0.793	1.728
K	0.837	—	—	—	0.234	0.512
P	0.532	0.03[23] 0.280[26]	0.272[26]	0.304[26]	—	—

Note: Number in parenthesis shows the No. of literature cited.

As seen in Table 16, the herring meat has comparatively abundant amounts of Na, K, P just as other fish meats. Comparing the amounts of inorganic compounds in the herring meat with the meat of other migrating fishes, recorded in columns 2 to 5, the amount of sulfur content is seen to be remarkably small, but this value is different from that of another investigator[24] who has reported 1.29% of sulfur content in the dried matter of herring meat.

As the inorganic components besides the components which are given in the Table, the amount of Cu has been stated to be 2.5 *mg/kg*[28], of fresh herrings meat, I is 2.1 *mg/kg*[29], As is 0.4~0.8 *mg/kg*[30], or 2.0 *mg/kg*[31]. According to For and Ramage[32], components, As, V, Cr, Mn, Co, Sr, Nb, Ag, Sn, Pb, were detected in the herring meal by spectrochemical analysis. According to Igarashi[10], in the air dried matter of herring meat (water content 7 ~ 8%), there are 0.67 ~ 0.72 % of sulfur, 0.03 ~ 0.28 % of phosphor, 0.28 ~ 1.15 % of calcium, 0.34 ~ 0.55 % of magnesium. He has learned that those components are more rich in the belly meat than in the back meat.

(2) *Sulfur components*

The sulfur compounds present in the fish meat are in both insoluble and soluble states. The insoluble part includes ether soluble and alcohol soluble sulfur. In this experiment the amounts of sulfur components were estimated in parts of water soluble, ether soluble, 95 % alcohol soluble, volatile, and insoluble. The results obtained are shown as in the 1st column of Table 17. (In Table 17, the numbers in parentheses show the percentage of sulfur in the total amount of sulfur.)

As seen in Table 17, about 22 % of the total sulfur in the herring meat was in water-soluble state, while the most part was in insoluble-state.

Generally speaking, the ratio of soluble sulfur to insoluble sulfur is about 1:2 ; this

Table 17. Sulfur compounds in herring meat and comparison with that of other fish meats (% of sulfur in the dried matter)

Sulfur \ Fishes	Herring	Tuna[33]	Salmon[33]
Total-S	0.061 (100%)	0.32 (100)	0.40 (100)
Water soluble-S	0.0132 (21.6)	0.12 (37.5)	0.13 (32.5)
Ether soluble-S	0.0025 (4.1)	0.20 (62.5)	0.27 (67.5)
95% alcohol soluble-S	0.0069 (11.3)		
Volatile-S	0.0102 (16.7)		
Insoluble-S	0.0282 (46.3)		

fact is observed in the tuna and salmon[33] meats as shown in Table 17.

(3) *Forms of phosphorus in the herring meat*

The greater part of phosphorus (60 ～ 75 %) in fish meat is inorganic form. The difference between the total amount of phosphorus and inorganic phosphorus is almost the same as the amount of lipoid-form, and there are only small amounts of protein form and organic phosphorus.

In this experiment, the amounts of various forms of phosphorus were estimated. The results obtained are shown in Table 18, lst column. (In Table 18, the numbers in parentheses show the percentage of various forms of phosphorus to the total amount of phosphorus in the herring meat.)

Table 18. Amounts of various forms of phosphorus in herring meat and comparison with that of other fish meat (% of P_2O_5 in the dried matter)

Phosphorus \ Fishes	Herring	Bonito[34]	Flat fish[34]
Total-phosphor	2.4402 (100%)	2.008 (100)	2.579 (100)
Alcohol ether-soluble-P	0.8146 (33.4)	0.546 (27.3)	0.639 (24.8)
Lipoid-P	0.7413 (30.4)	0.470 (23.4)	0.593 (23.0)
Water soluble-P	1.3286 (54.4)	1.302 (65.1)	1.928 (74.9)
Water insoluble-P	0.0733 (3.0)	—	—
Protein form-P	0.2970 (12.2)	0.033 (1.65)	0.065 (2.52)

As seen in Table 18, about 55% of the total amount of phosphorus in herring meat was water-soluble form phosphorus (inorganic, organic phosphorus), and other forms of phosphorus were 30 % of lipoid-phosphorus and 12 % of protein-phosphorus. When those results are compared with Takenouchi's results[34] which were obtained by the meat of bonito and flat fish, the herring meat is seen to contain a larger amount of protein-phosphorus than bonito or flat fish.

As the protein-phosphorus is present in the reddish meat ("Chiai") more abundantly than in ordinary meat[35], this fact obtained above, is probably due to the abundant amount

of reddish meat in the herring bodies.

2. Inorganic components in the blood

Blood was taken from 10 specimens of "Spring herrings" which were caught in early April (adult) (body length 29 *cm*, weight 177 *g* on the average). Twenty five *cc* of the isolated blcod was analyzed for the amounts of inorganic components. The results obtained are shown in Table 19.

Table 19. Amounts of inorganic components in the blood of herring

Components	*g*/25*cc*			*g*/1000*cc*
	Sample 1	Sample 2	Average	Average
K_2O	0.0070	0.0078	0.0074	0.296
Na_2O	0.0849	0.0855	0.0852	3.408
Cl	0.0751	0.0761	0.0756	3.024
CaO	0.0020	0.0024	0.0022	0.088
MgO	0.0009	0.0017	0.0013	0.052
P_2O_5	0.0177	0.0187	0.0182	0.728
Fe_2O_3	0.0160	0.0170	0.0165	0.660

As seen in Table 19, among the inorganic components in the herrings blood, NaCl is the principal. Other components were present in only small amounts.

VII. CHANGES OF THE AMOUNTS OF VOLATILE BASIC NITROGEN AND pH VALUE DURING THE STORING OF RAW HERRINGS MEAT

1. Amount of volatile basic nitrogen (V.B.-N) and pH value in fresh herring meat

The amount of V.B.-N and pH value were determined by employing the meat of fresh herring ("Fore-coming herring" caught off Yoichi). Those herrings were used immediately after catching for the determination. Back meat was taken from the herring bodies, and crushed through a chopper. To the crushed meat was added dist. water of 5 times amount of the meat, agitated thoroughly, and then filtered through the cotton. pH value of the filtrate was colorimetrically determined. The amount of V.B.-N was estimated by usual method (aeration method). Results obtained are shown in Table 20.

Table 20. pH value and amount of volatile basic nitrogen in fresh herrings meat

Sex	Body length	Body weight	pH	V. B.-N
♂	25 *cm*	123 *g*	6.8	7.15 *mg%*
♂	25	120	6.8	7.15
♂	25	120	6.8	7.15
♀	25	119	6.8	7.01
♀	25	119	6.8	7.01
♀	25	118	6.8	6.94

As seen in Table 20, there was no difference of pH value and amount of V.B.-N between male and female fish. pH value was 6.8 and the amount of V.B.-N was 7 *mg%*.

2. Changes of pH value and the amounts of V.B.-N in the herring meat during storing

(1) *Change of pH value of the meat stored in a closed vessel*

Twenty specimens of "Spring herrings" (adult) were caught, from which the back meat was taken and crushed. The crushed meat was stored in closed vessels in which the storing temperatures of 3° ± 2°C, 15° ± 2°C and 18° ± 2°C respectively. At definite intervals during the storing, samples of the meat stored was taken. pH value was determined by the same method as above described. The results are shown in Table 21 and Fig. 9.

Table 21. Change of pH value of the herring meat stored in closed vessels

Time (days) \ Temp.	3° ± 2°C	15° ± 2°C	18° ± 2°C
After catching	6.8	6.8	6.8
1/8 days	6.8	—	—
1/4	6.5	—	—
1	(in rigor mortis) 6.5	—	6.4
1¼	(softened) —	—	6.2
2	6.5	6.6	6.8
3	6.4	6.8	(bad smell) 7.0
4	6.4	(bad smell) 7.2	7.0
4½	6.0	—	—
5	6.4	7.2	6.9
6	—	7.2	6.9
6¼	—	—	7.4
7	—	6.8	7.6
7¼	—	—	7.8
8	—	7.0	8.0
9	—	7.6	8.0
10	—	8.2	8.2
11	—	8.2	8.2

As seen in Table 21 and Fig. 9, the pH value of meat changed gradually from 6.8 to 6.2 ~ 6.4 in 3 ~ 4 days stored at 3°C, or in one day stored at 15°C or 18°C. Afrerwards the pH value increased to alkaline side. With ascending of the storing temperature, the pH value showed larger. According to organoleptic observation, in the course of increasing of pH value, at pH 6.8, it has already smelled putrefactive odors. Therefore the limit of pH value of the herring meat which is edible was considered to

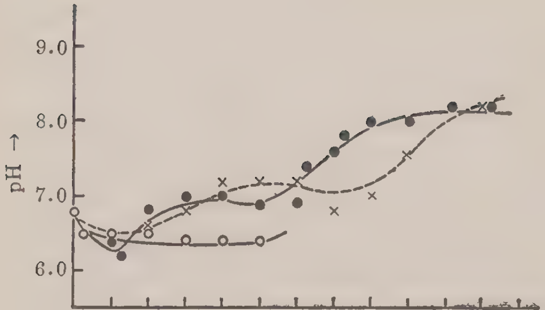


Fig. 9 Change of pH value of the herring meat stored in closed vessels

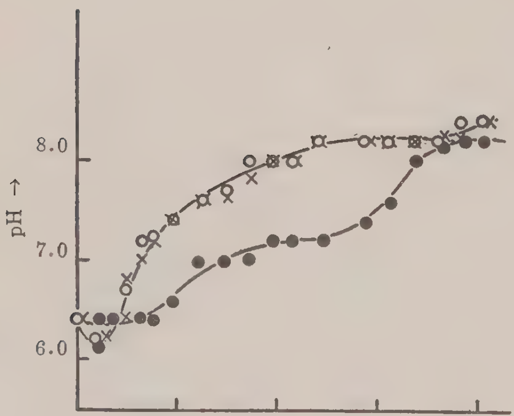


Fig. 10 Change of pH value during the storing of herring in open place

be below 6.5.

The results obtained above showed that lactic acid was produced in the herring meat by autolysis after the death, and thereby the pH value decreased at first; the pH value showed the minimum when the produced amount of lactic acid reached to the maximum. In the succeeding of bacterial decomposion, the amount of V.B.-N increased, and the pH value increased. Those results agreed with Macpheron's[36]. Yamamura[37] has estimated the relation between pH value and the amount of V.B.-N for 22 kinds of fish, and observed that the pH value was 6.5 at the incipient putrefaction of fish meat.

(2) *Change of the pH vlaue in the herring meat stored in open place*

Two specimens of male of "Spring herrings" (adult, 32 *cm* in length, 283*g* weight, 7 years old) which were caught off Yoichi and 2 females (31.5 *cm* in length, 311 *g* weight, 8 years old) were stored at 13° ± 2°C and 20° ± 2°C separatealy in round bodies. At definite intervals of the storing, the back meat was taken and the pH value was estimated by the same method as above described. The results obtained are shown in Table 22 and Fig. 10.

As seen in Table 22 and Fig. 10, there was no difference of the pH values between the male and female. Until the end of the initial 24 hours' storing, the pH value decreased, and afterwards the value increased comparatively rapidly with ascending of the temperature, in agreement with the observations reported in previous section (1). When the pH value showed 6.6 ~ 6.8 at the increasing period of pH value, the fresh bodies

Table 22. Change of pH value during the storing of herring in open place

Time \ Temp. / Sex	20° ± 2°C ♂	20° ± 2°C ♀	13° ± 2°C ♂
0 hrs.	6.4	6.4	6.4
18	6.2	6.2	6.2
24	6.2	6.2	6.4
42	6.4	6.4	6.4
48	6.7 (bad smell)	6.8 (bad smell)	6.4
65	7.2	7.0	6.4
77	7.2	7.2	6.4
96	7.4	7.4	6.6 (bad smell)
123	7.6	7.6	7.0
147	7.7	7.6	7.0
171	8.0	7.8	7.0
196	8.0	8.0	7.2
216	8.0	8.0	7.2
240	8.2	8.2	7.2
288	8.2	8.2	7.4
312	8.2	8.2	7.6
336	8.2	8.2	8.0
360	8.2	8.2	8.2
384	8.4	8.2	8.2
408	8.4	8.4	8.2

have smelled and became unedible.

(3) *Changes of pH value and the amount of V.B.-N in various parts of the stored herring body*

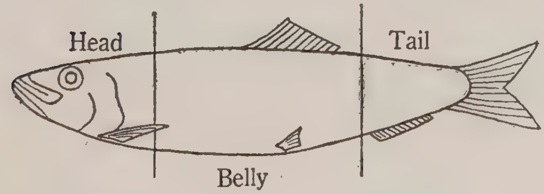


Fig. 11 Position of taking samples

From 5 male fish (248 *cm* in average length, 123.2 *g* weight, 6 years old) and 5 females (24.6 *cm* length, 117.6 *g* weight, 6 years old) of "Spring herrings",, head- belly- and tail-part meat were taken as shown in Fig.11. Meat taken from those parts was crushed and stored at 16° ± 3°C.

At definite intervals of the storing, samples from the various parts meat were estimated for the determination of pH value and the amount of V.B.-N. The results obtained are shown in Table 23.

There was no difference in the changes of pH value and V.B.-N between male and female. Therefore, those changes of male fish only are graphed as shown in Figs. 12 and 13.

Table 23. Changes of pH value and the amount of volatile basic nitrogen in various parts of the body of stored herring

Items / Time (hrs.)	Head-part				Belly-part				Tail-part			
	♂		♀		♂		♀		♂		♀	
	pH	V.B.-N (*mg%*)	pH	V.B.-N (*mg%*)	pH	V.B.-N (*mg%*)	pH	V.B.-N (*mg%*)	pH	V.B.-N (*mg%*)	pH	V.B.-N (*mg%*)
0	6.5	7.20	6.5	7.20	6.5	7.15	6.5	7.20	6.5	7.10	6.5	7.15
19	6.1	8.91	6.1	8.90	6.0	8.40	6.1	8.03	6.1	8.03	6.1	8.03
41	6.1	—	6.1	—	6.0	—	6.0	8.25	5.9	7.30	5.9	7.30
67	6.8	37.9	6.8	37.9	6.6	32.0	6.6	32.0	6.2	9.50	6.2	9.50
89	7.2	46.7	7.2	46.5	6.8	37.9	6.8	37.9	6.4	12.8	6.4	12.4
113	7.4	47.8	7.2	46.7	7.2	46.7	7.1	45.8	7.4	47.8	7.2	46.5
137	7.4	—	7.4	47.7	7.2	—	7.2	46.5	7.4	—	7.4	47.6
161	7.4	—	7.4	—	7.2	—	7.2	—	7.4	—	7.4	—
185	7.6	52.0	7.6	51.5	7.6	50.7	7.5	49.7	7.6	52.0	7.6	51.8
209	7.6	—	7.6	—	7.6	52.0	7.5	—	7.6	—	7.9	—
233	7.8	53.7	7.8	53.8	7.7	52.5	7.7	52.4	7.8	53.7	7.8	53.7
257	7.8	—	7.8	—	7.7	—	7.7	—	7.8	—	7.8	—
281	8.2	64.4	8.2	64.0	8.2	64.2	8.2	64.3	7.8	—	7.8	—
305	8.2	—	8.2	—	8.2	—	8.2	—	8.2	64.4	8.2	64.4
329	8.4	65.4	8.4	65.4	8.4	65.3	8.3	65.3	8.4	65.5	8.2	—
353	8.4	—	8.4	—	8.4	—	8.4	65.4	8.4	—	8.4	65.4
377	8.4	—	8.4	—	8.4	—	8.4	—	8.4	—	8.4	—
401	8.4	65.5	8.4	65.6	8.4	65.4	8.4	65.4	8.4	65.2	8.4	65.4

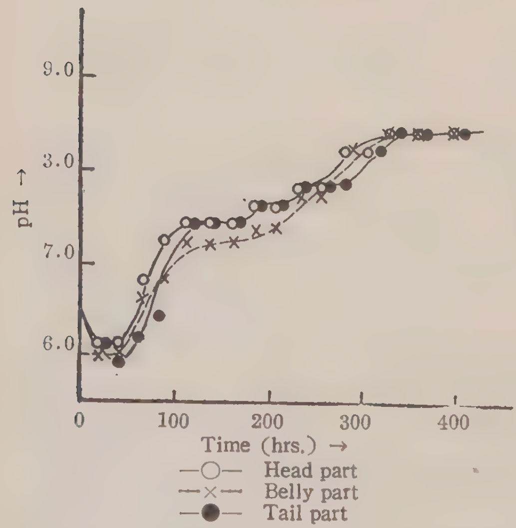


Fig. 12 Change of pH value in various parts of the stored herring body (16°±3°C)

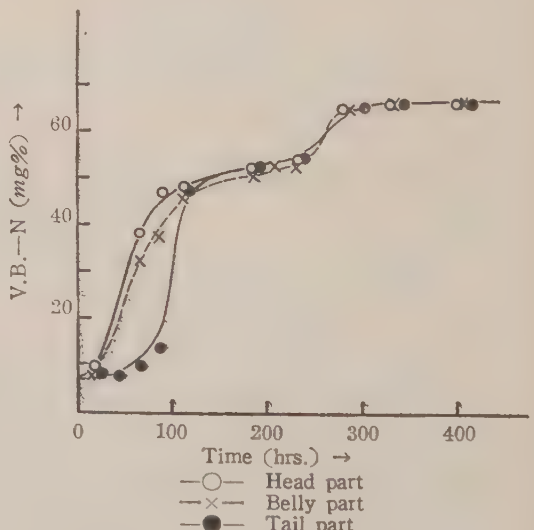


Fig. 13 Change of the amount of volatile basic nitrogen in various parts of the stored herring body (16°±3°C)

As seen in Fig. 12, the pH values of meat samples from various parts decreased once, and afterwards reached to about 6.0 in 24 ~ 48 hours; thereafter the pH values increased rapidly. After 100 hours' storing, the pH value became above 7.0. Afterwards the pH values gradually increased. To the initial increasing period of pH value, head, belly and tail parts reached slowly in order of time. There were remarkable differences of the pH value among the various parts of the meat, but after 100 hours' storing there was no difference.

The changes of the amount of V.B.-N shown in Fig. 13 indicate the relative relation with the change of pH value. The increasing of the amount of V.B.-N occurred late in order of head, belly and tail parts. From those results, the tail part meat is considered to be less decomposable than the head and belly parts meat. It is not yet known whether or not the difference of decomposable rate is due to the difference of chemical components. When the amount of V.B.-N reached 30 *mg%* in the meat at which point the incipient putrefaction begins, the pH value showed about 6.5. Those results agreed with the organoleptic results noted in the previous experiment.

3. Considerations

From the results obtained in this experiment, the pH value of the fresh herring meat was 6.8, and the amount of V.B.-N was about 7 *mg%*. When the raw herring meat is stored at various temperatures, with the lapse of time, the pH value decreased in the initial stage, and afterwards increased, while the amouns of V.B.-N increased. No difference of those changes showed between male and female fishes of herrings. Remrkable difference in those changes were observed between the places of the meat taken. The tail part meat of herring body was less decomposable than head and belly parts.

From these present experiments, the author could not determine the decomposability of herrings meat in comparison with other kinds of fish. But it has been said that herring meat is less decomposable. Prof. Tanikawa, is trying to verify the truth of that opinion by detailed experiments now, so he will report his results in future.

(The literature cited will be listed in continued paper of this series.)